JUN 22 1929

Vol. XIV JUNE, 1929 No. 9

The Journal of Laboratory and Clinical Medicine

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The Journal of Laboratory and Clinical Medicine

VOL. XIV ST. LOUIS, MO., JUNE, 1929 NO. 9

CLINICAL AND EXPERIMENTAL

A GROUP OF HIGHER BACTERIA FROM THE GENITOURINARY TRACT*

I. PRELIMINARY COMMUNICATION

BY SARA A. SCUDDER, B.A., AND DAVID L. BELDING, M.D., BOSTON, MASS.

THE incidence of nongonorrheal infections of the genitourinary tract is undoubtedly greater than ordinarily believed, but the difficulty of distinguishing between primary and secondary invaders renders the statistics unreliable. The common pus-forming bacilli and cocci have been reported in numerous cases, but the higher bacteria have not usually been associated with chronic irritation of the genitourinary tract.

Three strains of higher bacteria have been isolated from the genitourinary tract: strain A from the cervix of a child; strains B and C from the prostatic secretions of patients who had clinical signs of chronic urethritis, but no positive clinical or laboratory evidence of gonorrhea. The morphologic and biologic characteristics of these strains are recorded in order to facilitate their identification and later classification, since there is at present no universal agreement as to a satisfactory differentiation between the orders of higher bacteria. Their association with chronic irritation of the genitourinary tract and their superficial resemblance to streptococci make them of clinical as well as bacteriologic interest.

TECHNICAL METHODS

Clinical Material.—In obtaining the original cultures from the child, plates were streaked with secretions directly from the upper vagina and cervix. The cultures from the adults were made from fresh prostatic fluid kept at body

*From the Evans Memorial, Boston, Massachusetts.
Received for publication, August 2, 1928.

temperature until centrifugation. The medium on which the strains were isolated was veal infusion agar adapted by enrichment for the isolation of the gonococcus. In each instance the colonies on the original plates were numerous, plaque-like, and from pin-point to 1.0 mm. in size. Technical details have been previously described by Scudder.

CASE A.—This strain was isolated from a five-year-old girl who had been under observation since the age of four months with a diagnosis of malnutrition and rickets. At the age of seven months she received the additional diagnosis of gonorrheal vaginitis and during the next three years positive gonococcus smears were obtained from time to time. Between the ages of three and one-half and five and one-half years she was persistently examined for gonococci as a research case. During this period she had a mild intermittent inflammation of the urethra, cervix, and vagina, accompanied at times by a slight watery discharge. Smears showed consistently a lancet-shaped gram-positive diplococcus in pairs and short chains with rare pus cells, even when the clinical signs were least suggestive of gonorrhea. At times a gram-negative intracellular diplococcus, morphologically like the gonococcus was also present. Repeated cultures failed to yield gonococci. At the age of five a provocative injection of gonococcus vaccine slightly increased the clinical signs of inflammation and the smears from the cervix showed both gram-negative and gram-positive diplococci, but cultures yielded only pin-point colonies which proved to be a pleomorphic bacterium, Strain A.

CASE B.—A young man of twenty-six years with a history of repeated attacks of tonsillitis, was exposed to venereal infection eleven months previous to culturing. One month later he was operated upon for simple mastoid. Subsequently he noticed a scalding upon urination which was relieved by urotropin. Five months later a diagnosis of chronic urethritis, probably gonorrheal, was made. Occasional shreds in the urine and a morning drop were present, but there was never a definite discharge. Repeated smear examinations from the urethra and prostatic secretion failed to show gonococci. Cultures taken from a slight secretion obtained by prostatic massage gave no gonococci, a few colonies of staphylococci, and numerous delicate pin-point colonies of a pleomorphic chain organism, Strain B. Direct smears from the secretion showed pus cells and various gram-positive cocci with a few gram-negative diplococci.

CASE C.—A male patient, forty-eight years old, had a persistent intermittent discharge since an attack of gonorrhea twenty-eight years ago. An examination ten years ago revealed increased massage fluid and prostatic plugs, while smears showed numerous pus cells and a variety of unidentified organisms. During the last ten years no gonococci have been found in some forty examinations of the prostatic fluid, all of which were essentially the same, except for a gradual diminution of the amount of pus during treatment. At the time of isolation of Strain C the direct smear showed 50 per cent pus cells, many spermatozoa, and coccoid organisms in chains. In addition some of the leucocytes contained gram-negative bacteria morphologically resembling gonococci. Twenty-four to seventy-two hour incubation of streaked plates yielded only pleomorphic chain cocci in pure culture, which on further cultivation in broth proved to be higher bacteria.

Laboratory Methods.—Culture media of the hormone type were prepared with a minimum degree of heating and filtering. Adjustment was made with 10 per cent sodium carbonate, and the reaction was determined by means of Clark and Lub's technic with Sorensen's standard phosphates. The hormone media were used in the form of broth, 0.5 per cent agar and 1.5 per cent agar with and without defibrinated blood or other enrichment. Comparative fermentation tests, using nonsol glass tubes, were made with the following culture media: (1) hormone 0.5 per cent sugar free agar; (2) 0.5 per cent agar with peptone and standard amounts of sodium monohydrogen phosphate and potas-

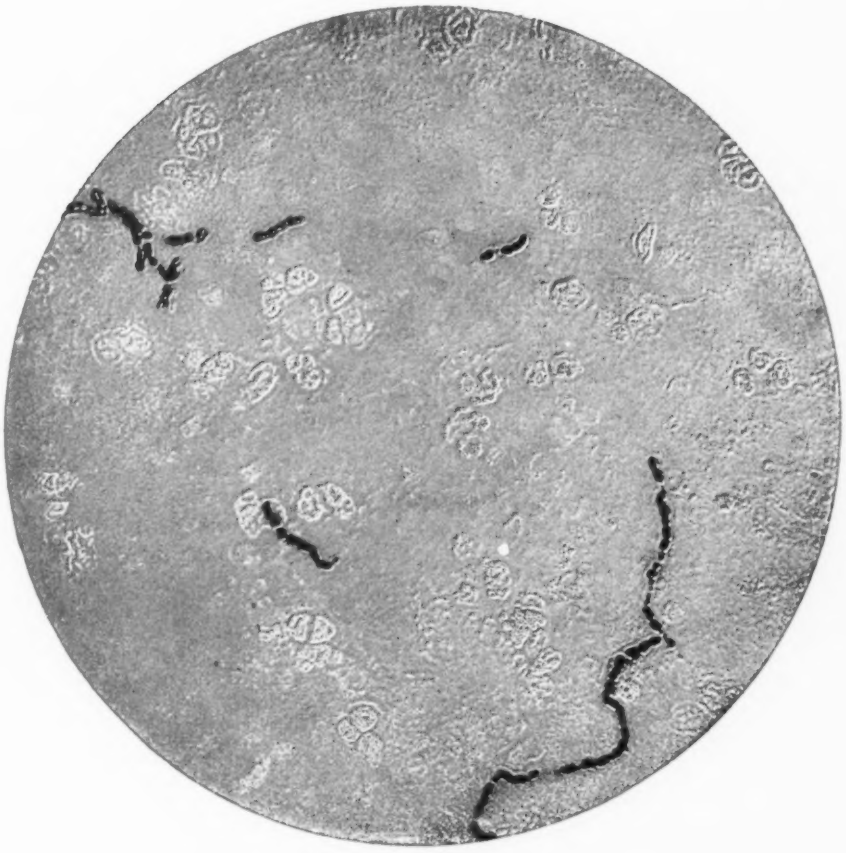

PLATE I, FIG. 1

PLATE I, FIG. 2

PLATE I, FIG. 3

Sperm culture of strain C, four hours at 37° C.
Fig. 1. Sheathed filament and lancet-shaped forms.
Fig. 2 & 3. Filaments which show dichotomous branching.

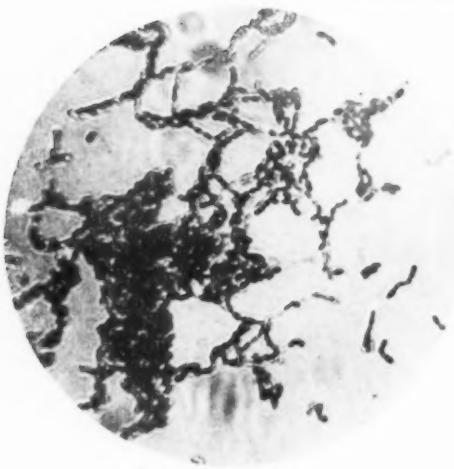

PLATE II, FIG. 1

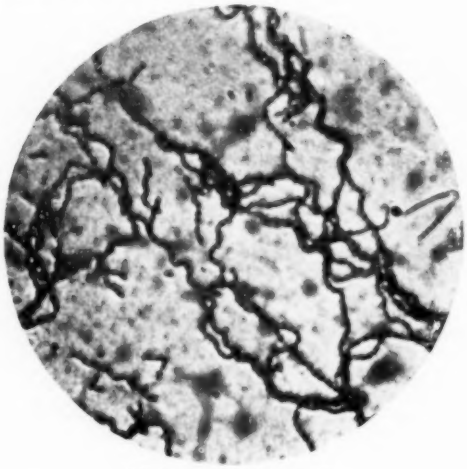

PLATE II, FIG. 2

PLATE II, FIG. 3

PLATE II, FIG. 4

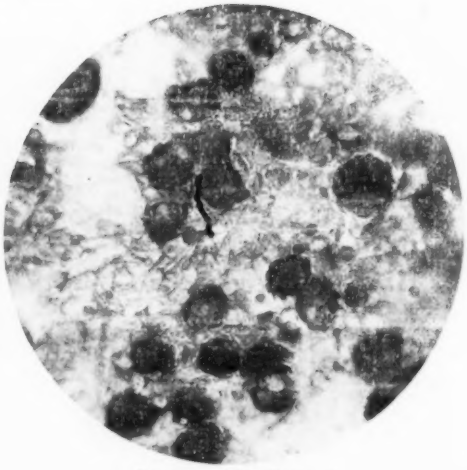

PLATE II, FIG. 5

Fig. 1-4 Twenty-four hour artificial cultures:
1 In water of condensation on veal agar slant; 2 and 4, in semi-solid agar; 3 in vitamine broth, marked parallel arrangement.
Fig. 5. Thread form in four hour sperm culture.

sium dihydrogen phosphate; and (3) potassium monohydrogen phosphate, peptone, and 0.5 per cent agar. The indicator of preference was bromthymol blue. Readings were made daily for one week, and acid production in twenty-four hour broth cultures was determined by titration. The cultural technic has been described by Torrey and Buckell.

The organisms were cultured in one per cent gelatin in hormone broth for seven days at 37° C. and were placed on ice daily long enough to permit setting of the gelatin. Indol, methyl red, and Voges-Proskauer tests were made as for fecal organisms.

The growth was studied at 37° C. in deep cultures, and in sealed hanging drop cultures on a warm stage microscope with which the organisms could be kept under continuous observation. As controls for the bacteriologic procedures and for comparative purposes the following groups of organisms were studied simultaneously: (1) three similar strains of higher bacteria from the respiratory tract, D, E, and F, (2) four strains of hemolytic streptococci, (3) one strain of *S. viridans*, (4) three types of pneumococci, (5) fifteen strains of *S. fecalis* isolated from polluted water and human feces, and (6) one strain of Actinomyces hominis (American Type Culture Collection).

The pathogenicity of the genitourinary strains was tested in mice, guinea pigs, and rabbits by peritoneal, intramuscular, and subcutaneous injections and by oral administration. The serologic relationship was studied by agglutination tests with immune sera for strains A, B, and C tested against the genitourinary and respiratory strains.

BIOLOGIC CHARACTERISTICS

Morphology.—In fluid cultures the organisms resembled long-chained, short-chained, and lancet-shaped diplococci. (Table I.) A conspicuous feature was the parallel arrangement of the long chains. (Plate II, Fig. 3.) Variation in morphology gave rise to transverse constriction and to irregularity in the size and shape of the separate protoplasmic elements. True (dichotomous) branching was noted occasionally. (Plate I.) Branched or extremely long filaments were rarely seen in smears made directly from the patient, the predominating form in the secretions being that of a diplococcus with pointed distal ends. In stained films a thin-walled sheath, prominent in hanging drop preparations, was vaguely outlined. (Plate II, Fig. 1.) Pleomorphism was a constant characteristic of cultures. Young cultures were fairly uniform; cultures more than twenty-four hours old contained many bacilli, diplobacilli, short chains, long chains, and tangled masses. Globoid bodies appeared as the culture aged, but were not seen to develop further. The filaments which appeared flattened with somewhat depressed centers, varied in width from 0.6 to 1.7 microns and in length from 2.2 to over 100 microns.

Growth on solid media gave a diplococcic type of organism with pointed distal ends in pairs and short chains. Chains of six and eight elements generally possessed one terminal element of oval shape and of larger size than the other elements. The sheathed filaments were positive with the alkaline modification of the Gram method of staining. The organism did not take the acid-fast stain. Often gram-negative and gram-positive elements appeared in

alternating sequences within the same filament. Likewise, the large terminal body at times appeared gram-positive when the rest of the filament appeared gram-negative.

The organism is nonmotile, although in hanging drop preparations changes in the position of the filament occur from time to time, which may be ascribed to motility or to passive movement by diffusion currents. The globoid and lancet-shaped bodies appear to have an independent motion.

TABLE I

CULTURAL CHARACTERISTICS

MEDIA		MACROSCOPIC	MICROSCOPIC
Meat infusion agar	With blood	Colonies pin-point, at first moist and semitransparent, later opaque and flattened, and green or rusty brown in color. Nonhemolytic. Strain C gives a small light halo. Discoloration of surrounding medium	Lancet-shaped diplococci in chains and pairs; frequently one terminal cell is larger than the other cells in the filament. Gram-positive in young cultures, becoming gram-negative within forty-eight hours
	Without blood	Similar to blood agar. Colonies greyish white, pin-point, becoming opaque with age; edges may be serrated and surface flattened. Maximum diameter 1.0 mm.	Similar to blood agar
0.5 per cent meat infusion agar		No surface growth, slight greyish growth below discrete with radiate outgrowths, 0.5 to 1.5 mm. in diameter. The greater the depth, the larger and more radiate the colony	Short and long chained diplococci. Pleomorphism noticeable, but not so marked as in broth cultures
Meat infusion broth	P_H 7.6	A web-like structure with long interlacing fluffy filaments suspended from meniscus or adherent to wall of tube until disturbed. Supernatant fluid clear after sedimentation. Strain C shows less tendency to web formation and slightly clouds the supernatant fluid.	Wavy bacilli, diplobacilli, and short chains in young cultures. Flattened filaments of marked pleomorphism and length, and individual globoid bodies in old cultures. Gram-positive young cultures becoming gram-negative after twenty-four hours
	P_H 6.8	Web formation less pronounced	Less marked pleomorphism. Filaments short, especially if broth is shallow and contains fermentable carbohydrates
Sugar-free infusion media		Negligible or slight growth	Usual pleomorphism
Potassium monohydrogen phosphate, peptone and dextrose		Negligible or slight growth	Usual pleomorphism

Cultural Characteristics.—In broth the growth appeared consistently as creamy white tufts adherent to the walls of the culture tube by means of delicate, trailing filaments (Plate 3). In deep broth culture, particularly at P_H 7.6, the growth formed as a delicate web-like structure which was suspended from the meniscus until disturbed, or until borne down by its own weight, leaving a clear supernatant fluid. Small pieces of sterile cork enabled these web formations to be more securely festooned. Although the supernatant liquid appeared clear to the naked eye, smears showed extremely small organisms in

chain formation. Transfers of the supernatant liquid to fresh broth gave rise to typical cultures. Strain C was less inclined to form webs and tended to cloud the supernatant broth, which is consistent with its shorter chain morphology.

In semisolid agar no surface growth occurred but growth was greyish and semidiffuse below the surface, and deep growth gave rise to discrete, radiate colonies. Facultative anaerobiosis occurred consistently.

On solid agar the colonies were plaque-like and ranged in diameter from 0.1 to 1.0 mm. The typical colony had a depressed center and a concentric ridge which appeared between the center and the periphery. The margin was

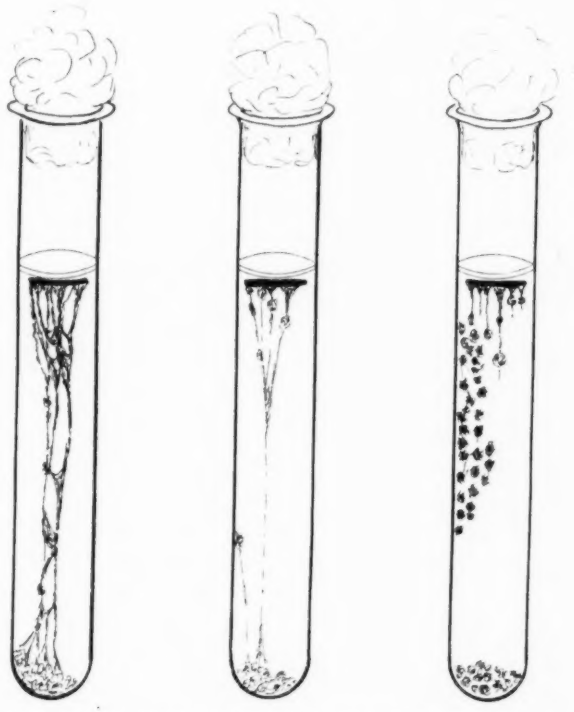

Plate III.—Strains A, B, and C. Broth cultures in festooned growth from pieces of sterile cork.

serrated and noticeably fringe-like when the surface of the medium was moist. Colonies which by chance grew between the solid agar layer and the sides of the glass tube or plate became scale-like and in the presence of blood had a dark color.

On blood agar the colonies appeared greenish and nonhemolytic, although Strain C produced a faint halo in the surrounding medium. The greenish color of the colonies was particularly pronounced if the blood was added to the melted agar at 60° C.

The genitourinary strains grew best at 37° C. No growth occurred at ice box temperature. Sluggish growth took place at 22° C., Strain C showing scarcely any growth. Death resulted at temperatures above 53° C. in ten

minutes. The optimum growth occurred at a hydrogen-ion concentration of P_H 6.9 to 7.2. Strains A and B gave a slight growth at P_H 6.1 to 6.3 and Strain C practically none.

In synthetic media, composed of potassium monohydrogen phosphate one per cent peptone and one per cent dextrose, no growth occurred within the first twenty-four hours with the genitourinary strains or with any control organisms except the fecal streptococci and *Actinomyces hominis*, which could be maintained in this medium for many generations. The organisms required the same selective media as the pneumococci and streptococci.

Physiology.—Table II gives a summary of the cultural and physiologic characteristics of our strains as compared with those of known chain cocci. None of the organisms produced indol, reduced nitrates, or gave a positive Voges-Proskauer reaction. The methyl red test was negative except for the fecal streptococci. Two strains of hemolytic streptococci liquefied gelatin. Our strains did not liquefy gelatin and, like the streptococci, they were insoluble in bile. They fermented dextrin, galactose, glucose, levulose, maltose, and saccharose. They did not ferment arabinose, dulcite, inulin, rhamnose, mannite, salicin, and xylose. Variable results were obtained with lactose for the genitourinary and with raffinose for the respiratory strains.

The type of fluffy web-like growth in broth, the radiate colonies in 0.5 per cent agar, the plaque-like colonies with fringing of the periphery, and the filamentous morphology differentiate them from the streptococci and pneumococci. The agar colonies are smaller, less dry, and do not have the yellow color or the mold-like appearance of the old *Actinomyces hominis* colonies. The genitourinary strains differ from the pneumococci by not fermenting inulin and raffinose, and from the fecal streptococci by failing to ferment arabinose, mannite, and salicin. In general the growth of streptococci and pneumococci was more diffuse in fluid media. The growth of all strains except the fecal streptococci and *Actinomyces hominis* was erratic in synthetic media containing standard amounts of sodium and potassium phosphate. Our strains differ from the enterococcus of French and German literature inasmuch as they do not ferment mannite and have a thermal death point under 60° C.

The fecal streptococci differed from the other streptococci, pneumococci, and our group of higher bacteria by (1) profuse growth in potassium monohydrogen phosphate with one per cent dextrose, (2) acid range, (3) rapid production of acid in dextrose broth, (4) positive methyl red reaction, and (5) fermentation of arabinose and mannite.

Bacteriologic and Serologic Grouping.—All attempts to show pathogenicity in mice, guinea pigs, and rabbits have failed, but the organism has been recovered from the heart's blood of mice twenty-four hours after intraperitoneal inoculation. Immune sera were produced in rabbits for the three genitourinary strains. Agglutination tests were made with the three sera, using as antigens strains A, B, and C isolated from the genitourinary tract; and in addition strains D, E, and F, isolated from the respiratory tract. Owing to the flocculent character of the growth, agglutination was difficult; but by using

TABLE II.

COMPARISON WITH STREPTOCOCCI AND PNEUMOCOCCI

	GENITOURINARY A B C	RESPIRATORY D E F	PNEUMOCOCCI	STREPTOCOCCI			ACTINOMYCES HOMINIS
				HEMOLYTIC	VIRIDANS	FECALIS	
Morphology	Pleomorphism	Pleomorphism	No pleomorphism	No pleomorphism	No pleomorphism	No pleomorphism	Pleomorphic
Growth in broth	Fluffy, web-like	Fluffy, web-like	Diffuse	Granular or diffuse	Granular or diffuse	Diffuse	Pellicle Discrete fluffy masses
Growth, semisolid agar	Radiate, fringing of periphery	Radiate, fringing of periphery	Diffuse	Granular or diffuse	Granular or diffuse	Diffuse	Radiate
Carbohydrate fermentation							
Salicin	–	–	–	Variable	Variable	+	
Mannite	–	–	–	–	–	+	
Raffinose	–	Variable	+	–	–	–	
Inulin	–	–	+	–	–	–	
Arabinose	–	–	–	–	–	+	
Bile solubility	–	–	+	–	–	–	
Growth in potassium monohydrogen phosphate	–	–	–	–	–	+	+
Methyl red	–	–	–	–	–	+	

the supernatant portion of rapidly-growing broth cultures, a uniform suspension was obtained. Serum dilutions ranged from 1:10 to 1:800. The approximate agglutination titer was 1:400.

Sera A and B agglutinated equally strains A and B but not strain C. Sera A and B agglutinated respiratory strain D, but more weakly than strains A and B, but did not agglutinate strains E and F. Serologically, genitourinary strains A and B were closely related to each other and were unlike strain C. Of the three respiratory strains, D showed some relation to A and B but none to C, while E and F were not related to A, B, or C. Two serologic types were present in the genitourinary strains and at least one other in the respiratory strains.

TABLE III

BACTERIOLOGIC AND SEROLOGIC GROUPING

STRAINS	AGGLUTINATION WITH IMMUNE SERA			CARBOHYDRATE FERMENTATION	
	A	B	C	RAFFINOSE	LACTOSE
Genitourinary					
A	++	++	−	−	+
B	++	++	−	−	−
C	−	−	++	−	+
Respiratory					
D	+	+	−	−	+
E	−	−	−	+	+
F	−	−	−	+	+

The serologic relationship of these strains did not correspond with their groupings by cultural characteristics. By direct agglutination the six strains fell into three serologic groups, (1) A B D, (2) C, and (3) E F, as compared with the bacteriologic groups (1) A C D, (2) B, and (3) E F. Strain D, which bacteriologically was classed with A and C, serologically was related to A and B. Strain C was sharply defined from A and B by cultural characteristics.

Identification.—A satisfactory identification of our organisms cannot be made owing to the prevailing confusion in regard to the classification of higher bacteria. They possess certain of the characteristics of the various groups which have been somewhat indiscriminately described as Cladothrix, Streptothrix, Nocardia, and Actinomyces. The generic name Cladothrix is in disrepute and the terms Nocardia and Streptothrix are under critical consideration. Our organisms are quite unlike the classical Actinomyces and Streptothrix, most of which give hardy, tenacious, and at times mold-like growth on solid media. They have certain points in common with the classical *C. dichotoma* of Cohn, though definitely at variance in others. Morphologically and culturally, particularly in respect to the plaque-like colonies, they seem consistent with Kligler's description of *Cladothrix placoides* isolated from dental caries.

The group within itself seems diversified, the individual strains varying widely in cultural and serologic characteristics. Further study of a larger number of strains may give additional information in regard to their generic relationship and to their association with pathologic processes.

DIAGNOSIS

The methods of differentiation between gonorrheal and nongonorrheal urethritis and vaginitis are time consuming. Bacteriologic diagnosis of gonococcus infection depends upon the identification of the gonococcus by morphology and arrangement in stained films of genitourinary secretions and upon positive cultures. The demonstration in films of the typical diplococcus of Neisser and the absence from culture of organisms which could be mistaken for it seem satisfactory evidence of gonococcus infection. Consistent negative results with carefully repeated smear and cultural examinations over a period of four to six months, especially when another organism predominates in the cultures, would seem sufficient to warrant a diagnosis of nongonorrheal infection. However, chronic gonorrhea during latent periods may give a picture of nonspecific infection with other organisms.

Baker claims that 15 to 20 per cent of genitourinary cases in private practice are of nonvenereal origin. Our observations suggest that about the same per cent may be true for vaginitis and cervicitis in children.

The mucous surfaces afford a favorable habitat for the higher bacteria. Several instances of pulmonary and general infection of Streptothrix or Nocardia have been reported. Kligler studied fifty-eight strains of *Cladothrix placoides* associated with dental caries. Bacteria, similar to our strains, have been found in the respiratory tract under normal and pathologic conditions.

The incidence in the normal mucosa of the genitourinary tract is unknown, since all organisms reported in the literature have been obtained from patients with pathologic conditions. Park and Williams recovered Nocardia from several cases of stillbirths with invasion of the placenta. Celler and Thalhimer described three strains of streptobacilli, repeatedly isolated from catheterized urine and associated respectively with chronic prostatitis, tumor of kidney, and edema bullosum vesicalis. Their organism, similar in many respects to our strains, was nonpathogenic for rabbits and guinea pigs, but was recovered regularly from the urine forty-eight hours after intravenous inoculations.

In order to obtain an approximate idea of the prevalence of these organisms in nongonorrheal infections, twelve patients with genitourinary inflammation without the presence of the gonococcus were examined as to causative organisms. Two gave our group of higher bacteria in pure culture, one hemolytic streptococci, three gram-positive bacilli similar to Döderleins bacillus, and six a mixture of bacteria, diphtheroid bacilli predominating.

The association of our organisms with chronic inflammation of the genitourinary tract raises the question of their pathogenicity. Although they appear incidental or secondary invaders rather than primary pathogens, chronic irritation seems to favor their existence. Two of our strains were obtained from patients with a gonorrheal history and one from a patient with urethritis following a probable streptococcic infection. Against pathogenicity are their absence in acute inflammation; the presence of similar strains in the respiratory tract, though usually from abnormal mucosa; and their nonpathogenic action in laboratory animals. In favor of their pathogenicity is their presence in practically pure culture in chronic inflammation long after the disappearance of the primary organisms.

SUMMARY

1. Three strains of higher bacteria possessing certain characteristics of the Cladothrix and Streptothrix of the early bacteriologists have been isolated from the genitourinary tract of patients with chronic urethritis. One of the three strains is culturally and serologically distinct from the other two.

2. Owing to the confusion in the classification of the higher bacteria, these organisms cannot be accurately placed in the present scheme of classification. Further study of many similar strains will be required before there can be a final identification of this group. The cultural and physiologic characteristics of three genitourinary and three respiratory strains are compared with various types of pneumococci and streptococci, in order to record their characteristics.

3. Their relationship to chronic inflammation of the genitourinary tract may be secondary and incidental, although there is some evidence to indicate that they prolong the chronic process.

4. In spite of their pleomorphic character, they may be mistaken in smear and isolation culture for streptococci. They are differentiated morphologically by the thin wall sheath.

5. Bacteriologic and serologic characteristics indicate that the group is composed of diverse strains. The bacteriologic and serologic groupings do not correspond. A serologic relationship was shown between a strain isolated from the respiratory tract and two from the genitourinary tract.

6. In comparative studies it was found that fecal streptococci are differentiated from these organisms and from other types of streptococci by the fermentation of arabinose and mannite, rapidity of growth in dextrose potassium monohydrogen phosphate with peptone, rapidity of acid production in dextrose broth, and a positive methyl-red test.

We are indebted to Dr. John Cunningham and Dr. Roger C. Graves of Boston for the use of clinical material from two patients, and to Mr. John A. Seaverns for the method of producing the colored photographic plates.

PHOTOMICROGRAPHIC TECHNIC

The photomicrographs accompanying this paper were made by John A. Seaverns of Boston. They are unusual in that they secure two-color separation with a single exposure. This method eliminates many difficulties and secures sufficiently good color value for classroom purposes.

The technic, which is quite simple, is as follows:

The original exposure is made with standard photomicrographic apparatus, using a panchromatic plate and a red filter. The negative thus obtained is printed by contact or enlargement on a lantern plate. When this latter plate is dried, it is used as a negative to print a second lantern slide and the pair so obtained bleached in a special bleaching bath and when thoroughly washed, stained with the regular stain used for the original micro slides. When dry, these two plates are bound with their film surfaces in contact, which, as they were printed one from the other, insures accurate registration without complicated technic. The original negative is preserved with care and

black and white lantern slides are made as usual. In making halftone printing plates, a pair of lantern plates are made but not bleached and are either used direct or as negatives for two glossy prints from which the halftone exposures are made.

This process, while not separating all the colors as well as the method of two separations from the original using selected green and red filters, makes slides which are, in general, satisfactory and avoids the extreme care necessary when two separations are made successively from the same subject, since it is difficult to avoid movement of the apparatus between exposures thereby causing lack of registration in the finished print. When using the two-exposure, two-filter method, the printing must be done in a projection apparatus in order that the two film sides of the compound lantern slide may be in contact and be registered. This two-exposure technic involves special apparatus and its accomplishment is extremely difficult since even slight differences in the thickness of the glass in the original pair of negatives will alter the registration. There are certain modifications in the filters which may be used for subjects having unusual color value and such filters are not difficult to obtain.

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THE SPECIFIC GRAVITY OF THE BLOOD: ITS CLINICAL SIGNIFICANCE*

By David Polowe, M.D., D.N.B., Paterson, New Jersey

THE clinical significance of the specific gravity of the blood has not received the recognition it deserves. In the falling drop method for determining the specific gravity of fluids, Barbour and Hamilton[1] have removed practically all the difficulties formerly associated with that determination. Only one drop of blood, obtained in the same manner as for blood counting or hemoglobin calculation, is required.

One can readily conceive that a knowledge of blood density in disease should be of equal, if not of greater, importance, as the routine determination of the specific gravity of the urine. The following investigation is, therefore, reported with the view of establishing the value of the routine determination of the specific gravity of the blood in disease.

Normal Densities of Human Blood.—Bamberger, cited by Lyonnet,[2] placed normal blood densities at an average of 10575 for males, 1053 for females.

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Received for publication, August 30, 1928.

Schmaltz[3] found them higher with males at 1059, females 1055, with afternoon densities lower by about 0.002. Leake, Kohl, and Stebbins,[4] using the falling drop method of Barbour and Hamilton, found the average densities for males to be 10565, for females 10533; with a maximum diurnal variation in the blood density of males being 0.0033, of females 0.0027. Polowe,[5] also using the falling drop method, made fifty-two observations on ten normal persons. He placed the average blood densities for males at 1057, females 1054, with the afternoon density drop averaging about 0.002. With these findings in mind normal blood densities for humans may reasonably be said to lie between 1050 and 1060, that they are higher in males, and higher in the morning than in the afternoon.

TABLE I

MISCELLANEOUS

OBSERVATION	BLOOD DENSITY	DIAGNOSIS
46	1032	Syphilitic stricture of rectum
62	1032	Severe Hg. poisoning (died)
48	1033	Pernicious anemia
30	1034	Tb. pleurisy with effusion
51	1034	Portal cirrhosis. Ascites
57	1035	Pancreatic Cyst
47	1037	Internal hemorrhoids
59	1037	Achylia gastrica with enteritis
102	1039	Enteritis of unknown origin
112	1041	Chronic myelogenous leucemia
74	1043	Pernicious anemia
4	1047	Rheumatic pneumonia
34	1047	Senility (88 yr. old)
67	1047	Perforated peptic ulcer
21	1051	Pneumoperitoneum
32	1051	Hypertrophic prostate, benign
73	1051	Osteosarcoma rt. mandible with metastasis to lung
104	1052	Duodenal ulcer
3	1054	Hemiplegia
23	1054	Head injury
83	1054	Pyopneumothorax with lung abscess
90	1055	Breast adenoma, benign
101	1055	Submaxillary adenitis
18	1056	Coma. Apoplexy
61	1056	Pernicious vomiting of pregnancy
84	1056	Obstructive jaundice
97	1057	Ulcer at pyloric end of stomach
105	1057	Syphilis
85	1058	Compound fracture of elbow
77	1061	Bronchial asthma

Clinical Blood Densimetry.—Copeman[6] cites Christison on the density of blood serum in early nephritis, when it is considerably lowered and may be as low as 1019, the normal specific gravity of the blood serum being about 1028. Schmaltz[3] concludes his extensive study of blood densimetry by stating that blood density varies within narrow limits in health and varies considerably under pathologic conditions. Lyonnet[2] cites Lloyd Jones' findings in nephritis, the blood density in parenchymatous nephritis being 1034, in interstitial nephritis 1062 to 1042. A differential diagnosis between cardiacs and nephritics is suggested in that the blood density in cardiacs is about 1059, in nephritics 1052. Bender and Polowe,[7] in a series of seventeen spinal anesthesias,

have found that with the fall in blood tension that follows the administration of the spinal anesthetic there is a concomitant fall in blood density. At the very beginning of the anesthesia there is a transitory increase in blood density. This is followed very soon by a small but definite fall in blood density. As the patient begins to react from the anesthesia the rise in blood tension is followed by an increase in blood density.

For the purposes of this study the blood densities of 85 hospital and clinic patients were arranged in ascending order. Twenty-four per cent of the blood densities were below 1040, 42 per cent below 1050. In the miscellaneous group (Table I), from which were omitted the cases of proved

TABLE II

CARCINOMAS

OBSERVATION	BLOOD DENSITY	LOCATION
88	1032	Gastrium
28	1035	Urinary bladder
8	1037	Pancreas
69	1039	Urinary bladder
58	1041	Peritoneum
115	1043	Rectum
81	1044	Colon
93	1044	Pancreas
116	1047	Peritoneum
114	1048	Submaxillary glands
100	1051	Bladder
65	1053	Lung
111	1053	Esophagus
96	1054	Cervix
87	1055	Cervix
71	1055	Esophagus
95	1055	Ali nasi
86	1055	Tonsil
106	1055	Breast
107	1056	Breast
91	1057	Breast
92	1058	Breast
94	1058	Tongue

TABLE III

CIRCULATORY, RENAL DISTURBANCES

OBSERVATION	BLOOD DENSITY	DIAGNOSIS
29	1029	Congenital atrophy of right kidney
52	1029	Chronic nephritis with death
55	1034	Pancarditis, death
5	1037	Acute arthritis with acute nephritis
76	1039	Pancarditis, death
54	1041	Cardionephritis
14	1047	Polycystic kidney
6	1051	Auricular fibrillation
56	1052	Myocarditis, death
24	1053	Chronic nephritis
78	1053	Low reserve kidney, eclampsia
53	1055	Myocarditis, kyphosis, death
82	1055	Aortic aneurysm
79	1056	Cardionephritis
98	1057	Subacute nephritis
70	1058	Myocarditis with heart block; chronic nephritis
103	1059	Ac. rh. fever with auricular fibrillation

carcinoma (Table II), of circulatory-renal disturbances (Table III), and of diabetes mellitus (Table IV); 30 per cent of the blood densities were below 1040, 50 per cent below 1050. Fourteen cases exhibiting fluid collections in tissues or serous cavities are listed in Table V.

Carcinoma (23 cases).—Carcinoma has long been a factor in the production of secondary anemia. The cases here reported were nearly all advanced cases proved by biopsy or necropsy. Ten, or 43 per cent, had blood densities below 1050. Of these, seven (70 per cent) obtained in carcinoma of the gastrointestinal tract or associated structures, the carcinoma of the stomach exhibiting the most marked grade of anemia. These findings are in keeping with those of Mayo[9, 10] who found that carcinoma of the proximal half of the colon tended to produce anemia more than any other part of the body, except carcinoma of the median portion of the stomach. The statistical work of Alvarez, Judd, MacCarthy, and Zimmermann[11] is in cor-

TABLE IV

DIABETES MELLITUS

OBSERVATION	BLOOD DENSITY	URINE DENSITY	GLYCOSURIA	INSULIN ADMINISTERED
17	1032	1020	yes	no
20	1039	1027	yes	yes
21	1040	1023	no	no
22	1047	1016	yes	no
16	1049	1020	yes	no
14	1051	1020	yes	yes
18	1053	1022	no	no
9	1053	1029	no	no
19	1053	1020	yes	no
8	1054	1020	no	yes
11	1054	1015	no	?
12	1054	1017	yes	yes
15	1055	1022	yes	no
13	1056	1025	yes	yes
10	1057	1037	yes	yes

TABLE V

FLUID COLLECTIONS

OBSERVATION	BLOOD DENSITY	LOCATION OF FLUID	DIAGNOSIS
51	1034	Fluid in abdomen	Portal cirrhosis
57	1035	Pancreatic cyst	Traumatic
9	1038	Pleural effusion	Tuberculosis
76	1039	Fluid in abdomen	Pancarditis
54	1041	Fluid in abdomen	Cardionephritis
58	1041	Fluid in abdomen	Carcinoma peritoneum
93	1044	Fluid in abdomen	Carcinoma pancreas
116	1047	Fluid in abdomen	Carcinoma peritoneum
45	1050	Edema extremities	Tricuspid insufficiency
56	1052	Edema extremities	Cardiac
65	1053	Pleural effusion	Carcinoma lung
79	1056	Edema extremities	Cardionephritis
105	1057	Edema extremities	Syphilis, late with diabetes mellitus
103	1059	Edema extremities	Acute rheumatic fever with auricular fibrillation and cardiac decompensation

roboration with Mayo in respect to anemias produced by carcinoma of the cecum and ascending colon. At Rochester, the belief is that the main factor in the production of anemia in carcinoma of the gastrointestinal tract is the presence of large ulcerated areas from which there is a constant oozing of blood and through which bacteria gain easy access to the body.

More recently, Alvarez and MacCarty[12] have shown that 92 per cent of gastric ulcers of a size less than a silver quarter are benign; those over a silver dollar in size are almost certainly malignant. One can readily see that there likewise may be a difference in the degree of anemia directly proportional to the size of the ulcer. While secondary anemias occur in benign as well as malignant gastric ulcer, the lack of anemia would incline one toward a diagnosis of benign ulcer. In other words, given a case in which the clinical history points definitely toward the presence of a gastric ulcer, the finding of a normal red blood count, hemoglobin, or blood density (or if these are only moderately reduced) would favor the diagnosis of benign ulcer.

Carcinoma of the urinary bladder seems also to produce a severe anemia, the blood density in two of the three cases reported being below 1040. Here again, perhaps, the ulcerated area offers a portal of escape of blood and of entrance to bacteria.

Attention is invited to the fact that, in this series, the more severe anemias, as evidenced by blood densities below 1050, occurred in carcinoma of hidden or not readily accessible areas (gastrointestinal tract, peritoneum, pancreas, urinary bladder). Carcinoma of other parts of the body did not seem to alter the normal blood density, even though most of the cases were quite advanced. This appeared to be particularly true of carcinoma of the breast and of the cervix.

All these facts should be taken into consideration when evaluating the presence of secondary anemia especially where the symptoms point toward involvement of the gastrointestinal tract.

Circulatory-Renal Disturbances (17 cases).—Forty-one per cent of this group exhibited blood densities below 1050. Seventy-one per cent of these were in cases having severe renal involvement and are in accord with the findings of Lloyd Jones, Christison, and Barbour[5] and Dawson. Barbour and Dawson state that the low colloidal pressure is reflected by a lowered serum specific gravity in cases of renal involvement.

Fluid Collections (14 cases).—Since specific gravity of the blood is closely bound up with water balance, it was considered profitable to study the blood density in cases exhibiting fluid collections in tissues and in the serous cavities. A very striking finding here, is the fact that eight cases (57 per cent of the total) had blood densities below 1050 and that all of these (100 per cent) presented free fluid in serous cavities. In only one case, pleural effusion in a carcinoma of the lung, was a blood density of over 1050 concomitant with free fluid in a serous cavity. In four cases the free fluids were exudates, three transudates, and two were not studied from this angle.

These findings would appear to predicate extraordinary changes in blood density before free fluid finds its way into serous cavities.

Diabetes Mellitus (15 cases).—Thirty-three per cent of the cases here presented exhibited blood densities below 1050. In 80 per cent of these, glycosuria was present. Glycosuria was present in only 60 per cent of the cases having a blood density greater than 1050. A further study of this phase of blood densimetry might bring some important light to bear on the relation of blood density to the renal threshold of sugar and to the dosage of insulin.

Comment.—Definite conclusions cannot be drawn from so small a series of observations. However, it appears likely that blood density determinations should be helpful in diagnosing such conditions as carcinoma of the gastrointestinal tract, cardiorenal disturbances, and water imbalances involving fluid collections in serous cavities.

It may be stated in closing that routine determinations of blood density in disease should be encouraged. Such blood density determination most certainly rounds out the laboratory picture, and in some clinical entities may be a definite guide toward accurate diagnosis.

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INFECTIOUS MONONUCLEOSIS*

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THE temporary increase of the large mononucleated cells of the blood (symptomatic mononucleosis) is observed in various diseases, as in malaria, varicella, German measles, kala azar, recurrent fever, amebic dysentery, streptococcus septicemia after angina, Vincent's angina, peritonsillitis, diphtheria, typhoid fever, typhus, acute pulmonary tuberculosis, lymphogranulomatosis, etc., and is well known (Merklen and Wolf, Moszcytz). Schilling also reports the occurrence of a monocytic phase between the leucocytic and eosinophilic phase in suppurative diseases. In more recent years cases on an apparently infectious basis were reported in which the main symptoms consisted in more or less generalized swelling of the superficial lymph nodes and an increase of the monocytes together with an absolute leucocytosis and absolute and relative lymphocytosis. As the nature of this peculiar blood reaction is still unknown and the number of reports published on this subject rather small, a brief discussion of the clinical symptoms and pathologic findings thus far known may be of interest, especially as several cases of this type of disease came to observation in recent months in Mercy Hospital.

HISTORICAL DATA

In 1907 Türk and later Marchand, Deussing, Jagic and Schiffner, Pribram and Stern, Haloi, Tody and Daniel described cases of a peculiar type of angina in which a mononucleosis and a swelling of the lymph nodes were the main characteristics. Schultz published in 1922 a series of such cases and called the disease "monocytic angina" or "lymphoid cell angina," which is probably identical with Pfeiffer's Druesenfieber (1889). The Americans, Sprunt and Evans and Bloedorn and Houghton, observed similar blood findings in patients with inflammatory processes of other organs and named the disease "infectious lymphomonocytosis" or "acute benign lymphoblastosis" respectively. Similar observations were made by others and diagnosed as "acute benign lymphatic leucemias" or infections with lymphatic blood reaction.

SYMPTOMATOLOGY OF MONOCYTIC ANGINA

The onset is rather sudden, starting with headache, malaise, chilly feeling or sometimes real chill, high fever of intermittent character, sore throat, which may also appear somewhat later, and dysphagia. There may be a generalized tender swelling of the superficial lymph nodes, especially the sub-

*Presented to the staff of Mercy Hospital, August, 1928.

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Received for publication, August 29, 1928.

mandibulary and cervical ones, which may vary in size from that of a bean to that of a date, rarely larger. The lymph-node enlargement may sometimes precede the sore throat. An edematous swelling of the face is also occasionally seen. The patient appears to be seriously ill.

The physical examination gives following findings:

Mouth: The tonsils are enlarged and covered completely or partly by a brownish to blackish coat which is usually restricted to these organs but which may sometimes extend to the uvula. In later stages dirty looking, suppurating, eroded surfaces are seen. Then tonsillitis is either of a lacunary or membranous type. Breath is fetid.

Neck: Swelling and tenderness of the lymph nodes.

Thorax: Normal findings.

Abdomen: Enlargement of the liver and spleen are usually observed. The spleen is firm but not tender. The swelling of the spleen may persist for several months after clinical recovery.

Jaundice is in general absent but occurs occasionally (Schmidheiny). Hemorrhagic diathesis of a generalized character is not present; scanty, scattered hemorrhages may exist. (Downey and McKinley, Schmidheiny, Baader, Schwenkenbecher).

LABORATORY FINDINGS

The bacteriologic examination of the throat by smear and culture shows the presence of streptococci and staphylococci and the frequent occurrence of a fusospirillosis. Diphtheria bacilli are always absent. The urine contains in general a small amount of albumin. Blood: A granulocytic leucocytosis sometimes exists for a short period in the beginning. It is followed by an absolute leucocytosis (usually 20,000-30,000, occasionally higher, up to 50,000 and 80,000) and an absolute and relative lymphocytosis and monocytosis (usually 6 to 9 per cent, but going up to 76 per cent), which is especially marked at the climax of the disease. This condition may prevail to a minor degree even during the period of convalescence, or the number of leucocytes may drop below the normal level and a leucopenia with lymphocytosis may be present (Weiss, Richards). The red blood picture is normal, and the number of platelets is either normal or increased.

Monocytes are large cells with a deeply basophilic cytoplasm, which is often vacuolated, and large, indented or lobulated, sometimes excentrically placed nuclei with a heavy chromatin network. Nucleoli are rarely present. The oxydase reaction is usually negative, but sometimes fine gray purple granules are observed instead of the coarse blue ones in granulocytes, or even a definitely positive result may be seen. Most of the authors agree that no conclusions upon the genesis of the monocytes can be drawn from the result of this test. The mononucleated cells of the blood are represented by the small and large lymphocytes, plasma-cells, lymphoblasts, myeloblasts and monocytes. The exact origin and characteristics of the monocytes is still an unsettled question. Three different tissues are named by workers on this subject as the possible sources of these cells which appear under various names in the literature (transitional cells [Ehrlich, Türk, Rieder], polyblasts [Maximow], leuco-

cytic cells [Mallory], adventitia cells [Marchand], lymphoid cells [Pappenheim]). The three sources from which monocytes may originate are:

1. The bone marrow (Naegeli).
2. The reticulo-endothelial cells (Schilling, Kaznelson, Mallory, McJunkin).
3. The lymphoid tissue (Arneth, Richards).

The monocytes of different origin differ from each other morphologically and functionally according to Moszczytz. McJunkin divides the monocytes originating from endothelial cells into two groups, those coming from the endothelium of blood vessels (hemendotheliocytes) and those from the reticulum of lymph nodes (lymphendotheliocytes). The lymphendotheliocytes show, according to this author, after supravital staining with neutral red a red rosette-like formation in the cytoplasm, while the hemendotheliocytes have a granular-hyaline appearance. Arneth asserts that the monocytes represent advanced developmental stages of large lymphocytes, while Richards believes that they are lymphoid cells of an immature type. The situation becomes still more complicated as there seem to exist transitions between lymphocytes, plasma-cells and monocytes (Schultz). Naegeli, however, claims that plasma-cells are either of lymphatic or myeloic origin, while Schultz and Arneth consider them as exclusively lymphatic cells on account of their negative oxydase reaction. In consideration of the different nature of the mononucleated cells of the blood Schultz classifies the anginas characterized by a mononucleosis into three types according to the type of mononucleated cell predominant at the climax of the disease.

1. Plasmacellular-lymphatic-lymphoid cell angina.
2. Monocytic angina.
3. Myeloblastic angina.

He notes that the first two mentioned types are the most important and frequent ones and believes that the plasmacellular type is probably only a morphologic variation of the monocytic type. He is not sure about the actual existence of the third mentioned type, which is clinically accompanied by a cutaneous purpura. But in the cases published by Hertz, Krumbhaar, Haken, Schwenkenbecher the majority of the monocytes showed a definitely positive oxydase reaction and Krumbhaar and Hertz designate these cells as myeloblasts.

Experimentally a temporary monocytosis can be produced by the repeated intraperitoneal injections of India ink, collargol, lipoids, proteins (Büngeler), trypan blue, sudan III, and other colloids and suspensions (red gold, lamp black) (Witts, Simpson, McJunkin and others). McJunkin effected a monocytosis by injections of tuberculous material into guinea pigs, also injections with smegma bacilli were successful. Sabin and Doan obtained similar results by injections of the phosphatid fraction of tubercle bacilli. The monocytes increased after repeated injections of these substances from the normal figure of 3 to 5 per cent to 10 to 15 per cent. Witts finally reported a considerable increase of the monocytes after a single injection of a vaccine of bacterium monocytogenes.

GENERAL ASPECTS OF THE DISEASE

Course: The course is protracted and extends over two or three weeks, and the symptoms decrease and disappear gradually (lysis). But also formes fruste are sometimes observed. There exists a tendency to recurrences. The monocytic angina affects mainly younger persons (ten to thirty years old) and occurs with equal frequency in both sexes. The prognosis is good; only very few fatal cases are on record (Haken). The disease seems to be contagious (Schittenhelm).

Treatment: The symptomatic treatment consists in the administration of antipyretics. Locally 5 per cent chromic acid was used. Arsenic compounds were given during the convalescence.

Etiology: The etiology is absolutely dark. While some authors consider the monocytic angina as a disease *sui generis* (Schultz, Schwenkenbecher), others are inclined to regard it as an atypical reaction of the hemopoietic system to an infectious agent of nonspecific nature. Weiss contends that there is no principal difference between an agranulocytic and lymphatic reaction, because he observed the transition of a lymphatic reaction into an agranulocytic one. The type of reaction is decided according to his statements by the virulency of the infective agent and the resistance of the body. A specificity of the infective agent is denied by him.

Differential diagnosis: The differential diagnosis includes the different types of tonsillitis. Only acute lymphatic leucemia needs special mention in this respect. The differential diagnosis may be difficult in the beginning on account of the leucemoid blood picture, but the presence of more mature types of lymphocytes in the blood and the invariably bad prognosis of the lymphatic leucemia beside the typical pathologic findings will always permit a correct diagnosis. In conclusion it may be mentioned, that the blood picture alone is not sufficient for the diagnosis of a monocytic angina. The other symptoms have to be considered too.

REPORT OF CASES

Mr. E. G. had two days before admission difficulties in swallowing which were soon followed by severe pains. The lymph nodes below the angle of the jaw became swollen and fever of moderate degree set in. The examination of the throat showed marked redness of the tonsils and buccal fossae. Dyspnea was temporarily present. During the course of the disease the patient developed a swelling of his face and was almost unable to swallow for several days. Throat smear showed staphylococci and streptocci in short chains.

Urine.—Albumin positive.

Blood.—6/23/1928. Erythrocytes 4500000, hemoglobin 85 per cent, leucocytes 5200. Differential count: neutrophiles 3, lymphocytes 54, monocytes 43.

6/25/1928. Leucocytes 9600, neutrophiles 18, lymphocytes 51, monocytes 31.

6/26/1928. Leucocytes 29400, neutrophiles 44, lymphocytes 24, monocytes 31, eosinophile 1.

6/27/1928. Leucocytes 7000, neutrophiles 62, lymphocytes 28, monocytes 8, eosinophiles 1, basophiles 2.

7/ 3/28. Leucocytes 7800, neutrophiles 31, lymphocytes 43, monocytes 22, eosinophiles 3, basophile 1.

Patient was dismissed on July 4, 1928, feeling well.

CASE 2.—Mr. D. C., sixty-five years old, had diabetes of three years' duration. He had developed about fifteen years previously a small pimple on the neck which extended con-

siderably in the following days. He entered the hospital for treatment of a carbuncle of the neck. The physical examination did not give any pathologic findings except those connected with the condition of the neck. There was no enlargement of superficial lymph nodes, and there were no cutaneous hemorrhages. The carbuncle was excised. Blood sugar on admission was 222 and dropped after insulin treatment to 133. The urine did not contain any sugar.

Blood.—4/17/1928. Erythrocytes 4220000, leucocytes 50600. Differential count: neutrophiles 10, large lymphocytes 87, monocytes 2, eosinophiles 1.

4/20/1928. Leucocytes 68800, neutrophiles 27, large lymphocytes 60, small lymphocytes 6, monocytes 2, eosinophiles 2, basophiles 1.

4/23/1928. Leucocytes 80400, neutrophiles 17, lymphocytes 79, monocytes 4.

4/24/1928. Leucocytes 87500, neutrophiles 21, large lymphocytes 74, monocytes 2, eosinophiles 3.

4/26/1928. Leucocytes 65600, neutrophiles 21, lymphocytes 76, monocytes 2, basophiles 1.

Roentgenogram of chest negative.

Treatment.—Radiation of the spleen.

4/27/1928. Leucocytes 12500, neutrophiles 16, small lymphocytes 35, large lymphocytes 34, monocytes 4.

5/ 1/1928. Leucocytes 25400, neutrophiles 20, large lymphocytes 30, small lymphocytes 34, monocytes 3, eosinophiles 2, basophiles 1.

5/ 7/1928. Leucocytes 25700, neutrophiles 33, small lymphocytes 40, large lymphocytes 34, monocytes 3, eosinophiles 2, basophiles 1.

5/12/1928. Leucocytes 14200, neutrophiles 40, large lymphocytes 16, small lymphocytes 40, monocytes 3, eosinophiles 1.

The oxydase reaction was positive in the monocytes.

Patient felt fine through the whole course of the disease and was dismissed when the ulceration of the neck was almost healed.

I am indebted for the history of Case 1 to Dr. F. Drennan and for that of Case 2 to Dr. W. Sullivan.

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AN EXPERIMENTAL STUDY OF THE VALUE OF MERCUROCHROME-220 SOLUBLE AS AN ANTISEPTIC AGENT*

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INTRODUCTION

IN ORDER for any antiseptic to be considered effective in surgical usage, it must kill pathogenic bacteria under the conditions existing in the tissues. It is equally important that the antiseptic accomplish this object without serious damage to the body as a whole or to its various organs.

The purpose of this communication is to record the results of experiments in which these two criteria have been applied to mercurochrome-220 soluble.† It is necessary therefore first to determine the tolerance of the tissues to mercurochrome and then to observe the effectiveness of a tolerated concentration of the antiseptic in the treatment of local infections.

The first of these objects has been accomplished by means of an original and unique technic for testing the absolute tolerance of tissues to any substance, first suggested by Dr. Barney Brooks, a technic which appears to reduce sources of error to a point as near the minimum as is humanly possible.

The determination of the tolerance of the tissues to mercurochrome involves two factors: the concentration used, and the time the tissues are exposed to the solution of the antiseptic.

EXPERIMENTAL

Tolerance of the Tissues to Mercurochrome.—Since the object was to determine the maximum concentration of mercurochrome and the time of exposure to this solution which the tissues could tolerate, it seemed necessary to find a means by which the tissues of a given part, an extremity, for instance, could be exposed to this when mercurochrome is injected intravascularly. If the mercurochrome is diluted by the volume of the circulating blood it is reasonable to assume that the concentration received by any given organ will vary with the richness of its blood supply. For experimental purposes intravenous injection of the drug has a further disadvantage in that its effects upon various organs are fused in a manner difficult to analyze. In order to obtain a true tissue tolerance it seemed best to isolate and empty temporarily the blood vessels of a part before filling them with mercurochrome.

We are indebted to Dr. Barney Brooks for suggesting the original technic which accomplishes this. It permits the retention of a known concentra-

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Received for publication, August 4, 1928.

†Hynson Westcott and Dunning.

tion of mercurochrome in the tissues for a definite time. The routine procedure employed in all experiments is outlined below.

METHOD

One of the hind limbs of a dog was shaved carefully from the level of the groin down. A compression bandage of thin rubber like that used as a dental dam was applied to the extremity from below upward, beginning at the claws, and extending to a level about 2 inches above the knee joint. A rubber tube was placed around the thigh to act later on as a tourniquet. In order to keep it from slipping down when later tightened, it was threaded under a stout cord, which snugly encircled the dog's body just above the iliac crests. With this special tourniquet in place but not tightened, the skin of the groin was sterlized in the usual manner with iodine and the animal anesthetized with ether. The femoral artery was exposed and lifted up by means of an encircling traction thread of silk. The tourniquet above was tightened until the pressure obliterated all pulsation in the artery. The bandage below was then removed, and a measured amount of mercurochrome at a given concentration and at body temperature was injected into the vessel until both it and the veins were filled under slight pressure.* By employing this method the mercurochrome may be retained locally as long as desired before sweeping it into the general circulation by removal of the tourniquet. Rigid asepsis was maintained throughout all the experiments.

Before beginning the actual tests of tolerance, the completeness with which the drug infiltrated the living tissues was determined. It was found that a few moments after the injection of 0.5 per cent mercurochrome solution pink splotches appeared in the skin which indicated the filling of the most superficial vessels. As the injection progressed the whole skin became uniformly stained. The knee joint of the animal was opened five minutes after the injection and both the joint fluid and the articular surfaces were found to be stained pink which indicated a complete infiltration of all the tissues of the extremity by the dye.

From the foregoing description we feel that the reasons why we adopted this method of testing tissue tolerance as an extremely fair and accurate one, are evident. It is simple and safe in application, avoids the unnecessary sacrifice of animals and yields end-points easy to interpret. Unlike general intravenous injection tests, it avoids the confusing pictures which may occur from the simultaneous effects of any drug upon several organs, or from its rapidly changing concentration due to excretion. Finally it possesses one feature which Claude Bernard always emphasized to his pupils as being very valuable. It permits easy simultaneous control experiments to be run upon the same animal.

The following is presented as an experiment which typifies the technic employed in all the determinations of the tolerance of the tissues to mercurochrome.

*The amount injected would vary in proportion to the size of the dog, but in each case the injection was continued gently until the artery would remain dilated with the solution and the pressure within it would cause a slight backflow into the syringe.

Experiment.—A dog was anesthetized with ether, and a roller bandage of rubber dam was applied with slight pressure from the toes up to the inguinal region on the right side. The femoral artery was dissected free and a fine hypodermic needle inserted into it. A tourniquet of rubber tubing was applied around the thigh just above the incision and the pressure bandage removed. Ten c.c. of 0.5 per cent mercurochrome solution warmed to body temperature were injected very slowly. There was an instantaneous appearance of the characteristic pink coloration in the skin, slightly mottled in distribution at first but soon evenly distributed throughout the skin surface and deepening in intensity.

Fifteen minutes after the injection the circulation was reestablished by removal of the tourniquet and the wound was then closed.

Result.—Twenty-four hours later the leg was somewhat swollen. Forty-eight hours after injection the mercurochrome was still slightly visible, and large dark sloughing areas were found on surfaces of both the thigh and the leg, but no generalized gangrene was present. The animal died the same day.

The results of 20 experiments, like the one described above, are summarized in Table I and Table II.

TABLE I

PRODUCTION OF GROSS PATHOLOGIC LESIONS BY HIGH CONCENTRATIONS OF MERCUROCHROME 220 SOLUBLE

TREATMENT		NUMBER OF ANIMALS TESTED	ANIMALS SHOWING GROSS PATHOLOGIC LESIONS (GROUP I)
CONCENTRATION OF MERCUROCHROME PER CENT	TIME OF EXPOSURE IN MINUTES		
0.500	15	3	3
0.500	5	2	2
0.350	15	1	1
0.350	5	1	1
0.130	15	1	1
0.065	15	2	2

TABLE II

TOLERANCE OF TISSUES TO MERCUROCHROME

TREATMENT		NUMBER OF ANIMALS TESTED	ANIMALS SHOWING GROSS PATHOLOGIC LESIONS (GROUP I)	ANIMALS SHOWING FUNCTIONAL EFFECTS ONLY (GROUP II)	ANIMALS UNAFFECTED (GROUP III)
CONCENTRATION OF MERCUROCHROME PER CENT	TIME OF EXPOSURE IN MINUTES				
0.014	5	1	0	1	0
0.0065	5	2	0	2	0
0.0033	10	2	2	0	0
0.0033	7	2	0	0	2
0.0033	5	3	0	1	2

From the results of the experiments on the tolerance of tissues to mercurochrome, it is apparent that the effects produced in the tissues by the different concentrations and times of exposure may be divided into three groups. Group I includes all gross pathologic lesions involving loss of tissue, such as localized or extensive gangrene. Group II includes mild effects functional in nature, involving no loss of tissue, but inconsequential changes, such as

slight edema, transient soreness, tenderness or limping. These effects were interpreted as indicating irritation caused by the weaker concentrations. Group III includes animals which showed no effects following injection.

Certain ones of the first group with gangrene died, and certain others were sacrificed to prevent unnecessary suffering. It was not thought important to express this in the tables, since we are here primarily concerned with local tissue effects.

From Tables I and II it may also be seen that the effects produced are the direct result of the concentration used; at all concentrations above and including 0.065 per cent, gangrene resulted from the injections; at 0.014 per cent, functional effects were produced; while a percentage of 0.0033 was tolerated perfectly in the cases of four out of five animals when it was retained for periods of five minutes, and in both animals tested at seven minutes.

As to the cause of the lesions in Group I of the first table the occurrence of gangrene might be thought to be caused by thrombosis due to intravascular clotting, the predisposing factors being the manipulation of the artery for injection purposes and the conceivable injuries of applying the bandage and tourniquet. Evidence is available that the technic is not responsible for the production of these lesions. In the first place no injury resulted in experiments with control legs in which normal saline was substituted for the mercurochrome solutions. In the second place at autopsy thrombosis appeared in only three dogs in the entire series in which gangrene resulted after mercurochrome injection. In these three the thrombosis occurred after the appearance of gangrene. The first case showed thrombi in all vessels three days after injection, the second a thrombus occluding the right femoral vein ten days after mercurochrome and the third same findings in both the femoral artery and vein nine days after injection. Thus from both the standpoint of negative control experiments and the rarity and late occurrence of thrombosis, it is evident that gangrene results from a diffuse effect of mercurochrome upon the tissue cells and not from primary thrombosis due to vessel injury.

In three of the experiments, isotonic salt solution was used as a diluent for the mercurochrome in place of the distilled water usually employed. The purpose of this was to see whether an isotonic solution would be less irritating as a vehicle for mercurochrome than one like distilled water which is hypotonic. No difference in effect was observed.

The element of time in influencing the results is important. Solutions retained fifteen or even ten minutes before release of the tourniquet, seem to permeate the tissues more thoroughly than those retained for five minutes. It may be assumed that the longer the mercurochrome remains within the artery at its full concentration the more tissue damage is accomplished. Thus concentrations of mercurochrome at 0.0033 per cent caused definite pathologic lesions when they were left in for ten minutes, whereas they failed repeatedly to cause the slightest pathologic change when left in for periods of five and seven minutes respectively. It may be stated then that the maximum concentration of mercurochrome tolerated by the tissues of dogs, when injected into the emptied vessels of an extremity, was found to be 0.0033 per cent, when retained for a period of seven minutes.

The following experiments show the effectiveness of mercurochrome under these tolerated conditions when it is used as an antiseptic in the treatment of abscesses, cellulitis, and gangrene of the extremities experimentally induced in dogs.

TREATMENT OF LOCAL INFECTIONS BY PERFUSION OF THE TISSUES WITH MERCUROCHROME IN DOSES OF MAXIMUM TOLERANCE

The experiments were planned to determine the effect mercurochrome has upon local infections of the extremities when the solution is injected by the technic described under the conditions of retention time and concentration tolerated by the tissues.

Method: Infectious Mixtures.—Equal quantities of clotted foreign (rabbit's) blood and eighteen-hour broth cultures of either Staphylococcus aureus or B. *histolyticus*, or the scrapings from the teeth of the dog were rubbed up in a mortar and used for inoculation.

Inoculation.—Both hind legs were shaved from the level of the groin down to the hock. About 2 inches above and medial to this joint, 0.5 c.c. of physiologic salt solution containing 1 per cent novocaine and 0.001 per cent suprarenin was injected subcutaneously into each leg. A small perforation of the skin was made at this point by cauterizing with the tip of a hot wire. Amounts ranging from 0.2 c.c. to 2 c.c. of the infectious mixtures were then injected either subcutaneously or intramuscularly through the perforation and downward to prevent drainage. When the dogs were inoculated subcutaneously, there was present beneath the skin a well-developed abscess within forty-eight hours; when inoculated intramuscularly either a marked cellulitis or localized gangrene occurred depending upon the nature of the inoculation used. No septicemia was observed during the course of these infections.

Treatment.—When the lesions in both legs had become pronounced, the blood was driven out of the extremities by compression bandages and a measured amount of 0.0033 per cent mercurochrome was injected into the femoral artery of one leg by the technic already described. The solution was retained for seven minutes before release of the tourniquet. The other leg, as a control measure, was not treated except for the application of the tourniquet bandage, and in a few instances the injection of isotonic salt solution in place of the mercurochrome.

Table III gives a detailed record of 12 experiments showing the character of the infectious material used, the amount and method of injection, and the lesions produced; the time after infection when the mercurochrome was injected and the amount used; and lastly a comparison of the progress of both the treated and untreated lesions over a period of three days from the time of treatment.

A comparison of the results of these experiments in Table III shows that of the 12 legs not treated with mercurochrome, 2 showed no change in condition, 4 were improving and 6 grew worse in twenty-four hours; of the 12 treated with mercurochrome, 1 showed no change in condition, 3 were improving and 8 appeared worse. At the end of seventy-two hours after the time of treatment only 3 of the 12 legs untreated were worse as against 6 of the

TABLE III

EFFECT OF A RESTRICTED PERFUSION OF MERCUROCHROME IN CONCENTRATION OF MAXIMUM TISSUE TOLERANCE UPON LOCAL INFECTIONS IN DOGS

INFECTION				TREATMENT MERCUROCHROME 0.0033 %			CONDITION OF LESIONS FROM TIME OF TREATMENT						VALUE OF MERCUROCHROME ON BASIS OF RESULTS	REMARKS
							UNTREATED LEG			TREATED LEG				
							HOURS			HOURS				
MATERIAL INJECTED WITH BLOOD	METHOD OF INJECTION	AMOUNT OF INOCULUM	NATURE OF LESION	HOURS AFTER INFECTION	AMOUNT INTO EACH LEG, C.C.	RETENTION TIME IN MINUTES	24	48	72	24	48	72		
Teeth scrapings	Intramuscular	2.0 c.c.	Cellulitis	24	17	7	W	W	SB	W	W	W	0	Killed on sixth day. Incision clean. Extensive cellulitis both legs
"	"	1.0 c.c.	"	72	15	7	W	B	B	W	W	W	0	Subsequent recovery
Pus	Subcutaneous	1.0 c.c.	Abscesses	48	15	7	W	W	W	W	W	W	0	"
"	"	0.5 c.c.	"	24	15	7	W	W	B	W	B	B	?	"
Staphylococcus culture	Intramuscular	2.0 c.c.	Cellulitis	24	15	7	N	N	B	N	B	B	?	"
"	"	2.0 c.c.	"	24	15	7	W	N	B	B	B	B	Plus	"
"	"	0.5 c.c.	"	72	15	7	B	B	B	B	B	B	0	"
"	Subcutaneous	0.7 c.c.	Abscesses	24	15	7	SB	B	B	B	B	B	?	"
"	"	0.5 c.c.	"	48	15	7	B	B	B	W	W	N	0	"
"	"	0.2 c.c.	"	48	15	7	N	B	B	W	B	B	0	"
B. histolyticus and staphylococcus	Intramuscular	0.5 c.c.	Gangrene	72	20	7	W	W	W	W	W	W	0	Killed on seventh day. Incision clean. Extensive gangrene both legs.
"	"	0.5 c.c.	"	24	20	7	SB	W	W	W	W	W	0	"

N—no change; W—worse; B—better; SB—slightly better.

treated. The treated and untreated legs showed no marked differences in the rate of their improvement. In one case only did there seem to be a positive advantage in the use of the mercurochrome solution.

Although the tissues of the infected extremities were perfused with a maximum tolerated concentration of mercurochrome, it is evident from a comparison of the results obtained that such a solution has no definite antiseptic value when thus employed in the treatment of experimentally produced local infections.

DISCUSSION

The original method described above in detail constitutes an excellent technic for testing tissue tolerance. While we have employed it only for testing the tissues of an extremity, yet it may be used for testing the tissues of any organ possessing a single blood supply. The injection can be interrupted at any moment in order to reestablish the current of arterial blood and thus to sweep into the general blood stream all the dye or other substance except that which has become fixed in the tissues. The resulting concentration in the blood stream is so small as to make its effect negligible. Thus the time of tissue exposure is controllable. The error due to tissue dilution may be assumed to be a constant one. Although we have only tested mercurochrome, yet the method lends itself readily to the testing of any substance.

So accurate are the end-points obtained by use of this technic that we feel it fixes a true tissue tolerance point for any substance.

It is interesting to compare the concentration produced in the blood stream by injecting the average dose of mercurochrome in an average man with the maximum concentration tolerated by the dogs in our experiments. Young and Hill[1] advocate an intravenous injection of 5 milligrams per kilogram of body weight in fulminating human infections. This in the average adult would amount to a dose of 30 c.c. of a 1 per cent solution. Regarding the total blood volume in the human as roughly one-thirteenth of the body weight, the vessels of a man of 150 pounds would contain 5230 grams of blood. If one introduces into this volume 30 c.c. of a 1 per cent mercurochrome solution, there would result immediately after injection a concentration of 0.0057 per cent. Our maximum tolerated concentration of 0.003 per cent approximates this when one considers how rapidly the strength of the mercurochrome must be reduced through the single factor of excretion when the dye is injected into the general circulation, a factor which is not present in the tests above recorded.

Concerning the treatment of local infections with mercurochrome, it may be said that the lesions produced were of a local character with no evidence of septicemia, and therefore were favorable for study by the particular method employed. The fact that the treatment was found not to be effective when applied to abscesses, cellulitis and gangrene of the extremities is conclusive proof that the highest possible concentration tolerated by the tissues was insufficient to check the progress of these experimentally produced infections.

[1]Young and Hill: Arch. Ped. 1927, xliv, 152.

SUMMARY

An original perfusion technic is described for determining the tolerance of the tissues of an extremity or of other organs to antiseptic solutions.

This technic has many advantages, chief among which are: (a) elimination of all constitutional reactions, (b) accurate end-points, and (c) easy performance of simultaneous control experiments.

Employing this technic in 20 experiments upon dogs, the maximum tolerance of the tissues to mercurochrome-220 soluble was determined to be 0.0033 per cent for exposures of seven minutes. A ten minute exposure to the same concentration produced gross pathologic lesions. Concentrations of 0.0065 and 0.014 left in for five minutes produced severe functional disturbances while those of 0.350 and above were found to produce gross pathologic lesions.

The antiseptic value of mercurochrome was determined by the treatment of experimentally produced local infections of the extremities with concentrations of the dye determined by the above technic to be the maximum tolerated by the tissues.

Eleven out of twelve local infections in dogs treated with mercurochrome under these conditions showed no definite therapeutic advantage in its use when the progress of the treated lesion was compared with that of a similar untreated infection.

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THE PATHOLOGY OF THE THYROID GLAND*

BY JOHN W. GRAY, M.D., NEWARK, N. J.

THYROID diseases are not common in the region where I have studied them, but I have reached certain conclusions which may be of value to other pathologists who work under similar conditions and are called upon to classify goiters and to correlate the laboratory and clinical findings.

I have found elaborate pathologic classifications confusing and unnecessary. The thyroid diseases which I encountered in examining five hundred specimens were classified as follows:

Diffuse colloid goiter		51
Adenomatous goiter		264
Simple	221	
Hyperfunctioning	43	
Cyst of the thyroid		2
Exophthalmic goiter		165
Chronic thyroiditis		3
Carcinoma of the thyroid		15

Diffuse colloid goiter is now rarely excised and most of the 51 specimens of this type which I examined were received several years ago. These

*Read before the Seventh Annual Convention of the American Society of Clinical Pathologists, Minneapolis, Minn., June 8, 9 and 11, 1928.

goiters were symmetrical and two to five times the size of normal thyroids. On sectioning I found they presented a granular or finely honeycombed appearance. Many contained multiple tiny colloid cysts and in a few there were large cysts which contained colloid or fluid substance. Microscopically the acini were irregularly distended by normal or degenerated colloid and the lining cells were cuboidal or flattened. Occasionally atrophic epithelial spurs projected into the acinar lumina, an evidence of preexisting hyperplasia. In several specimens hypertrophic areas were found throughout the gland. These areas either were remnants of the parenchymal activity which always precedes the development of colloid goiter or were a part of the hyperplastic reaction which may repeatedly occur in colloid goiter. In either case the cell hypertrophy was probably the result of iodine deficiency as shown by Marine[1] and was influenced by the demands of the organism for an increased amount of thyroxin. It is possible that in case the demands had been large and continuous a physiologic compensation in the form of colloid goiter would never have taken place and that the activity would have gone on until atrophy from exhaustion occurred. Colloid goiter, then, is a gland which has adjusted itself to carry on an increased amount of work without hypertrophy of the parenchyma cells or is a gland which is in a resting state following increased demands upon the organ.

More than one-half the goiters that I have studied were adenomatous in type. They were of particular interest because of their neoplastic character, their slow growth from abnormal structures, the great variety of growth and degeneration which they presented and because malignancy of the thyroid could frequently be traced to adenomatous tumors.

The adenomatous goiters were usually nodular, encapsulated and multiple. However, not infrequently, the growth was single and large in size. Occasionally the gland was symmetrically enlarged with small tumors embedded in the thyroid tissue. Several specimens were diffusely adenomatous, grossly resembling colloid goiter.

There are two types of adenoma, the fetal and the adult. The former contains no colloid and probably develops from so-called "fetal rests," the latter contains a large amount of colloid and develops from fetal adenomas and possibly from normal glands. The structure of the fetal thyroid consists of an undifferentiated mass of embryonic cells which later form acini. Up to puberty the interacinar parenchyma remains fairly abundant and even in adult life solid masses of cells persist. A compensatory growth of these "rests" may be initiated by hyperactivity of the gland but the growth is so slow that a nodule in the thyroid may be first noticed five or ten years later.

In adenomas studied by me, the fetal type predominated. It and the adult adenoma frequently occurred together in these goiters and both types of growth were also found in individual tumors. The typical fetal adenoma was sharply defined, grayish in color, dense in consistency and small in size. The adult or colloid adenoma was encapsulated, yellowish in color and soft in consistency.

The larger adenomas, because of inadequate and lacunar type of blood supply, showed gross evidence of hemorrhage and degeneration. This ranged

from slight central softening with small hemorrhages to large degenerative cysts with fibrous replacement and calcareous deposits. Cysts were single and multiple, small and large and contained semisolid or fluid products of blood, colloid and tissue degeneration.

Microscopically fetal adenomas were composed of tiny closely packed acini which contained no colloid. Others, which I inferred were of longer standing, showed all stages of acinar development. Acini of the typical adult adenoma were lined by multiple layers of small cuboidal cells and contained a normal amount of colloid. In colloid adenomas the acini became distended and, except for adenomatous areas, differentiation from diffuse colloid goiter would have been difficult. In some specimens adult acini were found in the center of the growth and fetal structures toward the periphery, rather positive evidence of the development of the adult type from the centrifugal growth of fetal adenoma. Slight or moderate lymphoid infiltration was present in a few adenomas. In degenerated tumors new acini had developed in blood masses forming secondary adenomatous growth, blood clot had undergone fibrous and hyaline transformation, and in rare instances epithelial structures had grown into cysts forming a papillary cystoadenoma. Blood pigment, degenerated colloid and cholesterine crystals were found in cysts, and in the fibrous walls of these cysts there were primary or regenerated adenomatous glands and calcareous deposits.

Adenomatous goiters were further classified in regard to their functional activity. Of 43 diagnosed as hyperfunctioning adenomatous goiters, 21 were so placed only through clinical data, while 22 showed cell hypertrophy either in the tumor or in the surrounding thyroid tissue or both. The clinical group could be explained only upon the assumption that there was an increased bulk of adenomatous secreting glands in such goiters. Pathologically the hypertrophic group could not be distinguished from exophthalmic goiter but clinical data again played a part in the classification, for although the basal metabolism was high in some of these cases none of them showed evidence of thyroid dysfunction.

A separate heading was made for cystic goiter because of two large thin-walled cysts containing clear fluid in which the absence of gland structure made it impossible to trace their origin.

The group of 165 exophthalmic goiters presented but few difficulties in the laboratory diagnosis. They varied in size from that of the normal gland to many times that size but for the most part were only moderately enlarged and retained the normal shape. They were firm in consistency. The microscopic findings were also characteristic. There was extensive epithelial hypertrophy. The acini were irregular in size and shape and were lined by a single layer of tall columnar cells which usually formed infoldings and papillary projections into the lumina. The cell nuclei were enlarged and many abnormally placed. This epithelial hypertrophy involved not only the acini but also the interacinar parenchyma cells. Some glands contained no colloid while others contained varying amounts. The microscopic picture was changed by the administration of Lugol's solution, the cells were less hypertrophic, colloid was more abundant and some specimens were so altered that wide areas

resembled colloid goiter. Although it was impossible to estimate the degree of hyperthyroidism in such glands, it was always possible to establish the diagnosis through persistent hypertrophic areas. In almost all of the exophthalmic goiters the vascular stroma was infiltrated by lymphocytes and in some of them there were lymphoid deposits containing germinal centers.

An explanation of the etiology of exophthalmic goiter is not simple and is largely hypothetical. This thyroid disease is not common in some countries where goiter is most common, which suggests that the individual reaction as well as the stimulus should be considered. Psychic shock immediately preceded the onset of exophthalmic goiter in several cases which I have observed. Plummer[2] advanced an attractive theory of hyperfunction plus dysfunction as the cause of Graves' disease. In support of this two-product theory Haines[3] reported a case in which myxedema was produced by the administration of Lugol's solution and the phenomena of exophthalmic goiter existed when it was not administered.

Three of my specimens were classified as chronic thyroiditis because inflammatory processes predominated. The reaction consisted of lymphocytic infiltration and fibrous production. In two the parenchyma was definitely atrophic, and they represented to me the atrophy of exhaustion following continuous hyperfunction of the gland. The slight evidence of inflammation found in colloid and adenomatous goiters, the marked lymphocytic infiltration so common in exophthalmic goiters, and the productive changes in "burned out" goiters, indicated a relationship of stimulus, hypertrophy, and atrophy which might be explained through infection as suggested by Cole and Womack.[4]

Carcinoma occurred in fifteen, or 3 per cent, of my thyroid cases. Seven were malignant adenoma, 2 papillary adenocarcinoma, one scirrhous carcinoma, and 5 undifferentiated adenocarcinoma. The malignant adenomas were encapsulated and solid or solid and cystic. In two specimens of early malignancy the gross appearance was that of fetal adenoma. The structure was essentially adenocarcinomatous although great variation occurred in the development of acini and wide undifferentiated areas resembled solid carcinoma. Of the two papillary adenocarcinomas one developed in a large cyst while the other was a nodular tumor. The latter was of more malignant grade than the former as shown by the history of rapid growth and by the microscopic examination. In the former neoplasm the papillary and alveolar structures were for the most part lined by columnar cells while in the latter the cells were polyhedral in shape, contained large hyperchromatic nuclei, and undifferentiated cell groups invaded the stroma. The scirrhous carcinoma was a very firm irregular growth which had been present for several years and was adherent to the surrounding tissues. Microscopically it resembled scirrhous carcinoma of the breast and the extensive fibrosis had apparently acted as a protective factor in the malignant advancement of the tumor. The 5 undifferentiated adenocarcinomas were types frequently termed solid carcinoma. Four of these were single firm tumors of considerable size, the fifth was small and hard. They were composed of solid groups of atypical, deeply stained cells which infiltrated the capsules. Metastasis occurred in 4 of these cases.

It was impossible to determine whether these undifferentiated carcinomas originated in adenomatous or normal thyroid tissue. The grade of malignancy of my thyroid carcinomas, except the scirrhous type, paralleled the degree of differentiation of the tumor cells.

In this attempt to simplify the pathologic classification of thyroid diseases, I should suggest in conclusion that our understanding of thyroid pathology demands special standards dependent upon the development, structure and function of this highly specialized gland, and that the wide variations in the functional activity and structure of the gland in different physiologic states may explain variations which occur in different parts of this and other countries.

DISCUSSION

Dr. Wm. C. MacCarty.—As one of the founders of this Society I want to say something to those who have been presenting papers. Two gentlemen apologized a little bit. I used to go to a certain society where there were older members present and was almost afraid to call my soul my own. Dr. Gray happened to think how many goiters I had seen, and he apologized. I want to leave this little tradition with you. The Society was for the purpose of the young man who thinks. It makes no difference whether he had one case or fifty thousand, let him think about what he sees; let him draw his conclusions and come here and speak them. I never shall forget how uncomfortable I have been in certain meetings. I think that is one of the reasons I helped organize this one. We want to encourage young people. This is a young people's Society. We want that one person that is right. We will never get that if we apologize and if older people are given preference. I have seen in the paper the elaborate preparations made for flights across the Atlantic. I feel this very much myself. I still feel uncomfortable in the presence of that old Society. Don't ever let us get into that state of mind.

Dr. Philip Hillkowitz.—We frequently get thyroids for diagnosis in the laboratory, and we are often at a loss for proper classification. We are not able to pigeonhole them so easily as they are in the textbooks. I should like to get some suggestions from Dr. Gray as to how he classifies them. There is a need for a uniformity of terminology among pathologists. What constitutes chronic tonsillitis or chronic appendicitis? I do not like the term toxic thyroid. I cannot tell whether it is toxic or not. I would be very glad to hear a discussion on the part of practical pathologists as to how they solve the question.

Dr. George T. Caldwell.—In some of the exophthalmic goiters there is a diffuse infiltration of lymphoid cells. I was surprised to find that this proliferation of lymphoid tissue became the predominant feature to such an extent that the epithelial cells in many of the acini were completely replaced by lymphoid tissue. One could feel sure this was lymphoid tissue from the presence of definite lymph follicles. The cells in the central portions of these follicles are larger and more lightly stained. In some of these thyroids the epithelial structures are nearly completely replaced by these large lymph follicles. Along with the disappearance of the epithelial cells, however, there usually goes a very marked proliferation of fibrous tissue of the gland. There has been relatively little said about these thyroids in which the epithelial structures are replaced by lymphoid tissue. I wonder if they are common in Dr. Gray's section of the country.

Dr. C. I. Owen.—I had the same problem that these men have been having. I worked with a thyroid surgeon. Between the two of us we developed a classification we both understood. We do not recognize Graves' disease; we call that marked hyperplasia. We recognize colloid goiter which would not be operated; the adenomas of colloid type; the fetal type; the various tumors and inflammations. As for the lymphoid proliferation, I see that quite commonly, being in the thyroid belt. It is found in people who are members of the status lymphaticus group.

Dr. Robert A. Keilty.—The generally accepted surgical classification of thyroid glands from the pathologist's point of view is rather unsatisfactory. The term "toxic adenoma" from the clinical viewpoint may adequately meet the needs of the surgeon but when carefully studied pathologically, these glands may show no evidence of tumor but a well marked reaction explained on inflammatory grounds. There is a great need for a revision of thyroid nomenclature with a more careful and critical study of the actual pathology present in the glands fitted to the clinical picture. This is especially true in the nodular types of thyroid which are so universally called toxic or multiple adenomas when pathologically they show none of the structure of tumor as we understand it elsewhere in the body.

In 1921 I briefly reported bacterial findings in a series of thyroid glands removed surgically and since that time I have studied the question from the inflammatory and focal infection viewpoint. The presence of foci of round cells in the interacinal structures is, I believe, an inflammatory manifestation of bacterial reaction in many cases. This corresponds to the lymphocytic hyperplasia of chronic inflammations and as such many thyroids are to be considered from the standpoint of chronic thyroiditis.

The surgical removal of the thyroid gland per se is not by any means the last word in goiter therapy.

Dr. John W. Gray (closing).—I thank Dr. MacCarty and others for their discussion of this paper. Although conclusions from a limited amount of material may be of value I should emphasize the importance of first becoming familiar with extensive authoritative studies on the subject. In answer to Dr. Hillkowitz' question regarding classification I agree with Dr. Owen that it should be as simple as possible, that it is much easier to correlate the laboratory and clinical findings under a few headings than otherwise. I admit that exophthalmic goiter is not a scientific pathologic term, nor is it a good clinical name since it combines two symptoms of a disease both of which may be absent. However, if exophthalmic goiter and toxic adenoma are more readily understood by the surgeon than parenchymatous hypertrophic goiter and hyperfunctioning adenomatous goiter, I have no objection to using such terms. Dr. Caldwell's question regarding the classification of goiter showing extensive lymphoid infiltration is easier to answer than to explain. It is commonly present in exophthalmic goiter but seldom present in adenomatous goiter. In three of my specimens the lymphoid elements predominated to such an extent that a diagnosis of chronic thyroiditis was made. Dr. Keilty touched upon infection as an etiologic factor in the production of goiter. I mentioned the preliminary report of Drs. Cole and Womack and consider it the most important contribution we have on the subject.

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HENOCH'S PURPURA DUE TO FOOD ALLERGY

By I. S. Kahn, M.D., F.A.C.P., San Antonio, Texas

ABOUT a month after the inception of the study of this case, my attention was called to a report of three similar cases by Alexander and Eyermann.[1] The subject and its literature are so well covered by them that further comments would be superfluous beyond adding the report of a confirmatory case of this rather unusual, puzzling clinical entity with the obvious suggestion of the necessity of study from an allergic point of view of chronic hemophilia and purpura.

Mrs. L. M. J., aged forty-nine, married twenty-five years, no pregnancies, gave a negative family history except that one nephew was a "bleeder." No known allergy in the family.

Past History. Acute nephritis twenty years previously requiring medical supervision about one year. No recurrence. Two uterine curettages seventeen and fifteen years ago for fibroids and polypi. No recurrences. The operations were done to correct prolonged and excessive menses. Menstrual history normal the past fifteen years and never affected by the present condition. Had one attack of intercostal neuralgia with herpes twelve years ago. Severe urticaria persisted through one summer thirty years ago and again twenty-two years ago. There have been a few attacks since of no great severity. The patient has never noticed any connection between the urticarial and the purpuric eruptions. No other illnesses have occurred except occasional acute infections of the upper respiratory tract. No hay fever or asthma. She has always been susceptible to bruising from slight traumatism. Has always had frequent nosebleeds of considerable difficulty to control. Gums have always bled easily. Two teeth extractions, however, were done within the last four years with little or no difficulty from this source.

Present History. The first eruption of "black and blue" spots occurred on the upper arms and thighs eight years ago, lasting two weeks with exacerbations and retrogressions. Since the initial attack, a few new "black and blue" spots have recurred every three or four days on arms, thighs and lower legs, only once on the face and rarely on the abdomen or chest. The largest number of comparatively large areas, varying in size from that of a silver dollar to the palm of the hand, remembered at any one time, was about twenty. Individual purplish areas have usually lasted a week or ten days, then fading to yellow, disappearing completely in about a month's time. She has never had any joint symptoms, but has usually been able to foretell an eruption by noting a few hours or days of muscular soreness where the eruption would subsequently appear.

Since the initial eruption, nosebleeding has been very frequent, occurring at times daily and occasionally two or three times daily. Three years ago there were daily nasal hemorrhages over a two months' period. On one occasion she bled for six hours. For the last three years, she has had nosebleeds of considerable severity at least twice a week. There has been one aural hemorrhage, six years ago.

Digestive disturbances have occurred for twenty or twenty-five years, taking the form of intense abdominal pain and vomiting. No diarrhea, but considerable belching. There was never any jaundice. The digestive disturbances would usually be accompanied by either nosebleeds or the black and blue skin eruptions, or by a few urticarial lesions.

The initial etiologic diagnosis of the nasal hemorrhages as purpuric was made by Dr. W. A. Hitchcock, a nasal specialist of Boston, some four years previously. Several thorough gastrointestinal examinations have been negative.

This case was first seen by me October 10, 1927, the morning after a severe nasal hemorrhage, accompanied by a few scattered urticarial wheals and a few small petechial areas on the arms and legs. General physical examination was negative. Nasal examination by Dr. C. J. Boehs was negative. Special examination by Dr. W. E. Nesbit, including blood, was negative.

On account of the urticarial history and the fact, that at that time it was noticed that a number of my seasonal hay fever patients were showing bruised areas from trifling traumatisms, an observation since not infrequently confirmed in my experience, the possibility of an allergic basis was at once considered. Skin tests by the cutaneous method over a number of days were then done to approximately two hundred antigens. During the course of testing, intradermal tests were done to the ragweeds and grasses, using 1:50 extracts, securing definite positive reactions to the grasses. These special pollen tests were followed almost at once by generalized itching over the entire body lasting for twenty-four hours, but not producing any other symptoms or any skin eruption. Skin tests were slightly positive to onion, shellfish and the split proteins of wheat.

Subsequent History. Since October, 1927, she has been under the following antiallergic precautions: First, restriction of wheat and cereals, except for toasted bread; second, avoidance of fish and onion. Since this type of diet was suggested, the patient has noticed that either skin eruption or nosebleed or digestive disturbances, or all three, can be brought on by eating flour-thickened gravies, sauces and soups, and she herself now considers this factor, and onion her principal trouble.

At this writing, March, 1929, this case has been under observation one and a half years. There have been in this period six small nasal hemorrhages of only a few moments' duration, about six light attacks of urticaria of a few hours' duration, and almost none of the petechial eruption. These relapses have invariably followed unavoidable breakage of the ordered dietary restrictions. Previous to this study, this case had always been a heavy bread-eater and a good liver generally.

About the end of April, 1928, she had three or four severe digestive disturbances lasting several days, accompanied by considerable loss of weight, a few hives, two small epistaxes and a considerable amount of purpura, one attack ascribable to the usual thickened flour gravy, the other three attacks being due to Boston brown bread. Abdominal pain was intense in each instance, simulating typical acute cholecystitis. Gastrointestinal examinations at the time at the Winchester, Massachusetts, Hospital by Drs. R. G. Vance and M. J. Quinn were negative.

This patient was seen again February 22, 1929, reporting continued adherence to dietary restrictions without medication of any kind, and absolute freedom from previous symptoms during the past nine months, the longest period of well-being in twenty-five years.

She was retested at that time to all common foods, showing slight reactions to banana, strawberry, lamb, crab, halibut, mackerel, codfish, pear, sweet potato, and a strongly positive reaction to peanut, which she knows annoys her. Definite purpuric areas from 3 cm. to 5 cm. in diameter were noted from six to eight hours later, surrounding the skin tests to plum, barley, halibut, mackerel, lima and string bean, probably traumatic and not specific. At no time did her actual etiologic factors show any but the slightest possible positive reactions.

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ASTHMA DUE TO HOUSEHOLD ARTICLES*

Report of Nineteen Cases Due to Dusts from Mattresses

By Milton B. Cohen, M.D., Cleveland, Ohio

ALTHOUGH it has long been recognized that the majority of asthmatics have symptoms due to hypersensitiveness to some substance, the ingenuity of the physician is frequently taxed to the utmost to determine the origin and nature of the exciting agent. Innumerable substances have been implicated from time to time. Many of these arise from animal emanations and from the products used in the manufacture of various household articles. Kern[1] first called attention to house dust as a cause of asthma, and Cooke[2] demonstrated sensitivity to dust in over 40 per cent of patients whom he tested. Rowe[3] found 42 per cent of one hundred and sixty-two patients sensitive to house dust extracts, and felt that this substance, house dust, was frequently of importance as a cause for symptoms.

For the past three years, it has been our custom to test all patients with extracts made from dusts collected from various articles in their environment. The dusts are collected as follows: The cloth bag is removed from a vacuum cleaner; the cleaner is operated for a moment to free the working mechanism of any dust which may be present, and a sixteen pound paper sack is tied on in place of the cloth bag. A sample of dust is then taken from the mattress of the patient's bed; at least a teaspoonful is required. The bag is removed, labeled; a new bag is tied on, and another sample of dust is obtained from the pillows. Other samples of dust are collected from each mattress and set of pillows, from each rug, from the overstuffed furniture, and from any other articles in the home which may harbor dust. Each sample is placed in a wide-mouthed bottle and covered with Bernton's fluid at room temperature for forty-eight hours. The extracts are filtered and are used by the scratch method.

Among two hundred patients who have been tested to dusts collected and prepared in this manner, nineteen have been found who gave large reactions to extracts of the dust collected from their own mattresses, but who did not react to cotton or kapok, of which the mattresses were made. In each instance complete relief was produced almost like magic by discarding the offending mattress and the substitution of a new one. However, in three cases attacks recurred after free intervals of from four to six months, whereupon positive skin tests were again obtained to extracts of the new mattresses. It was found however, that recurrences could be prevented if the mattresses were covered with some impervious material, either rubber sheeting or Dupont's satin fabricoid.

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Received for publication, September 19, 1928.

Studies are being made to determine the nature of the sensitizing material in these cases. It is probable that mattresses become infected with molds to which patients become sensitized, as has been pointed out by W. Storm van Leeuwen.[4] This is probably what has occurred in these cases, since mattresses, the dust extracts of which give no reactions and which produce no symptoms in patients when new, do cause trouble after several months' use. The following cases illustrate these points:

N. B., a married woman of thirty-four years, has had asthma of a perennial type for the past fifteen years. During this time the longest free period was a six months' period which was spent away from home. There were no clues in the history pointing to the etiologic factors.

Examination revealed a well developed woman of thirty-five years with moderate chronic bronchitis and slight emphysema. Blood and urine studies revealed no abnormalities. Routine skin tests, using food, epidermal protein, pollens, and sachets, two hundred tests in all, were negative. A reaction was obtained to an extract of dust from the mattress on which she slept. No reaction was obtained to cottonseed, or to kapok.

Treatment consisted in discarding the offending mattress and completely renovating the bedroom. This was done on January 29, 1927. The attacks ceased within a week and have not recurred to date, seventeen months later.

J. E., a boy of thirteen years, has had attacks of asthma occurring about once a month for seven years. There had been eczema during infancy, and frequent attacks of bronchitis with wheezing, beginning in early childhood and continuing until the onset of the asthmatic attacks. The longest period of freedom from attacks was two months, but there was some rhinitis almost daily.

Physical examination revealed no intrinsic causes for the attacks. Routine skin tests using 200 substances revealed positive reactions to several fruits, to beef, and to spinach. Removal of these foods from the diet had no effect on the symptoms. Tests with autogenous dust extracts revealed a strongly positive reaction to the dusts from the patient's mattress and from a davenport in the living room.

The substitution of a new cotton mattress for the old one, after a complete renovation of the bedroom, produced relief from the asthma for a period of six months. During this time there was no rhinitis.

After the six months' interval, sniffling and other symptoms of allergic rhinitis recurred, and were followed, within two weeks, by a mild asthmatic seizure. A rechecking of the environment revealed a hypersensitiveness to dust collected from the new mattress.

This mattress was covered with rubber sheeting to preclude the escape of dust. No further attacks have occurred during a period of observation of one year.

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AN UNTOWARD EFFECT OF BARIUM CHLORIDE IN PRODUCING SHORT RUNS OF ABERRANT VENTRICULAR BEATS*

By Thomas M. McMillan, M.D., and Charles C. Wolferth, M.D.,
Philadelphia

FOLLOWING its introduction into the therapeutics of A-V heart-block by Cohn and Levine,[1] barium chloride has come to be used fairly extensively to increase the idioventricular rhythm of this disturbance and so to prevent the occurrence of syncopal and convulsive seizures.

Its clinical use was suggested by the results of animal experiments by Rothberger and Winterberg[2] in a paper in which they showed that the drug increased the irritability of the ventricles as shown by the occurrence of isolated ectopic ventricular beats and even short runs of paroxysmal tachycardia.

Among those who have reported upon the use of the drug in clinical heart-block, in addition to Cohn and Levine[1] are Herrmann and Ashman,[3] Heard, Marshall and Adams[4] and Strouss and Myer.[5]

On the whole these reports are favorable toward beneficial effects of the drug. Its use, moreover, has been thought to be entirely safe within the limits that the drug is usually prescribed. In one of their cases Herrmann and Ashman[3] used five times the usual therapeutic dose with no other untoward results than nausea and vomiting.

In a recent experience with barium chloride we encountered an untoward effect to which we feel attention should be called; the more so because the same disturbance occurred in the case of Strouss and Myer,[5] although they did not stress its potential danger. The disturbance to which we refer was the occurrence of runs of irregular ventricular tachycardia, a mechanism not far removed from ventricular fibrillation.

A résumé of the essential history and course of the patient follows:

A white woman, fifty-three years old, had been under our observation, either as a private or hospital patient, continuously for six years. During this time she had had five attacks of complete heart-block, none of which lasted for more than two months. During the periods of slow rhythm she was breathless on the slightest exertion, very easily fatigued and showed a slight but generalized edema. Between these attacks she went for periods longer than a year with normal sinus rhythm and without disturbance other than slight fatigue and breathlessness on exertion. Examination showed only moderate evidence of circulatory disease except for the periods of heart-block. The peripheral arteries were only moderately sclerosed, though the blood pressure was constantly elevated, the systolic varying between 168 and 145, and the diastolic between 100 and 80. The heart itself was no more than slightly enlarged, the left base being 12 cm. and the right 3 cm. and the poststernal dullness 6.5 cm. Auscultation revealed only a systolic apical murmur. At the apices of both lungs there was an old inactive tuberculous process.

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Received for publication, September 7, 1928.

The first three attacks of heart-block disappeared spontaneously after having lasted various lengths of time up to two months. In the fourth attack (January, 1927) she was given barium chloride for the first time and left the hospital after ten days with a normal sinus rhythm on a maintenance dose of 20 mg. twice daily. On this she continued to have a normal sinus rhythm with the exception of some brief periods of 2:1 block, lasting for only a few minutes or, at most, hours until eight months after her last discharge from the hospital when she developed complete A-V heart-block which continued until her death in December, 1927.

During this last period of heart-block, while still under the care of one of us in her home, the dose of barium chloride was increased to 20 mg. four times a day. After three days this resulted in the appearance of a very disturbed ventricular rhythm, which, without an electrocardiogram, was interpreted as being due to short paroxyms of ventricular tachycardia and led to the discontinuance of the drug. On December 5, 1927, after hospitalization had been instituted, the drug was again begun in a dosage of 20 mg. four times a day and continued for four days. It was then discontinued because of the return of frequent short periods of a very disturbed and irregular ventricular tachycardia (Fig. 2). The disturbance was present in about the same degree on the day after the drug was discontinued. On the second day occasional isolated extrasystoles were still noted, though no runs were present. Almost exactly seventy-two hours after the barium was discontinued, the patient died very suddenly. Two hours before death the pulse rate was 36 and its rhythm regular.

ELECTROCARDIOGRAMS

Fig. 1 shows the three customary leads of an electrocardiogram taken the day before barium chloride was begun. This tracing shows complete A-V block with a usually regular idioventricular rhythm of approximately 28 beats per minute.

Depressions of the ventricular rate occurred quite frequently, bringing the rate down to 16 beats per minute for a few cycles. These alarming depressions of rate suggested the likelihood of the occurrence of periods of ventricular standstill with accompanying Stokes-Adams seizures. These never occurred (unless her sudden death was the result of such an attack), but the fear of them was one factor that led us to use barium chloride.

After barium chloride had been exhibited for twenty-four hours, the alarmingly long pauses between ventricular beats became more frequent, and even the regular and more dominant ventricular rate became slower, being 28 before and 25 after twenty-four hours of barium therapy. The auricular rate became quickened from 78 to 93 after barium. We do not regard the change in either the auricular or ventricular rate as being a barium effect but rather as a coincidence.

The first three strips of Fig. 2 are the customary three leads taken after forty-eight hours of barium. They show the auricular rate quickened to 126 and the dominant ventricular rate increased to 30, which is slightly higher than the prebarium rate, though there are still periods in which the latter becomes quite slow. It also shows single ventricular extrasystoles coupled with preceding supraventricular impulses.

Fig. 2, lower three strips, shows the electrocardiogram taken after 220 mg. of barium chloride had been given in ninety-six hours. It shows the idioventricular rhythm being frequently interrupted by runs of as many as seven ventricular extrasystoles occurring at a rate of approximately 210 per minute. These beats, while apparently originating at the same focus, are quite irregu-

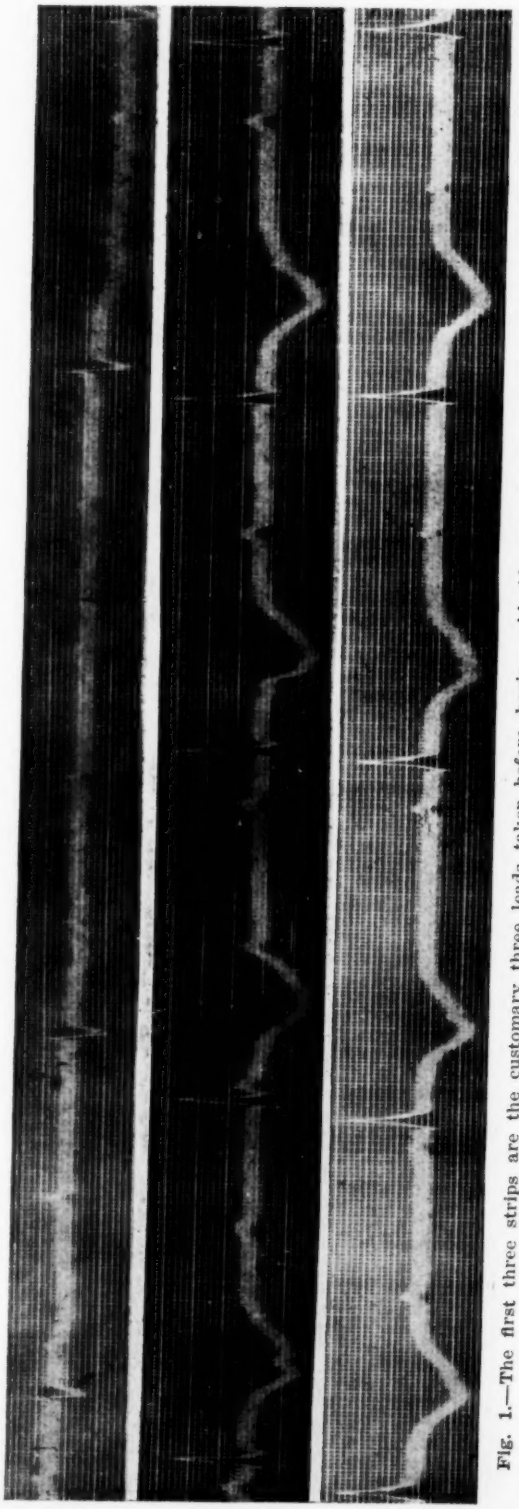
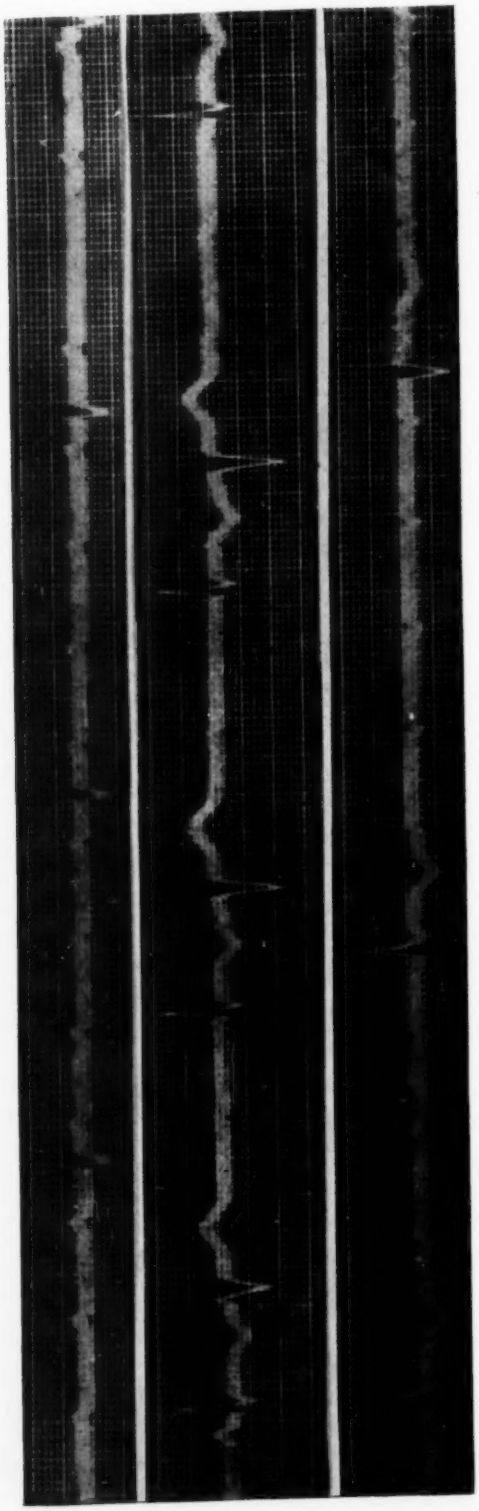


Fig. 1.—The first three strips are the customary three leads taken before barium chloride was begun. There is complete A-V heart-block. Time intervals one-fifth and one-twenty-fifth seconds.



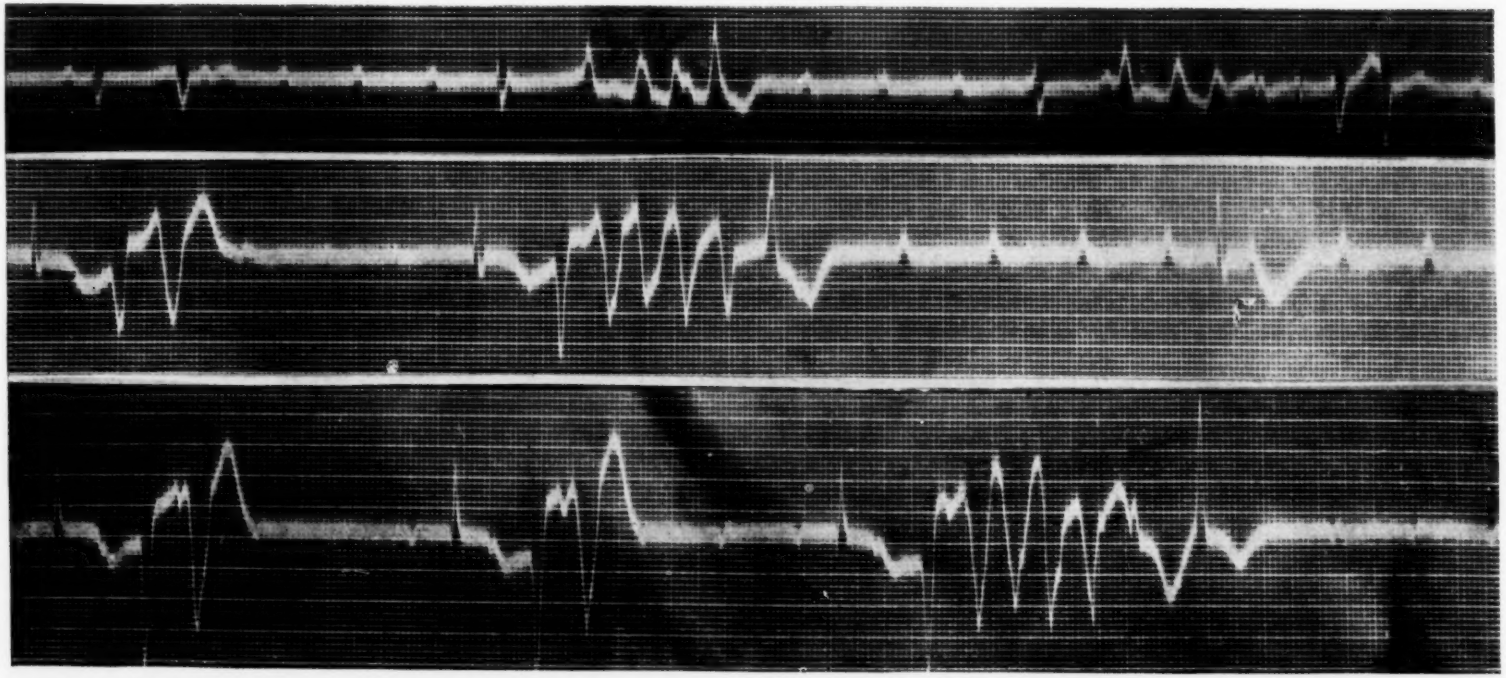


Fig. 2.—Top three strips are the tracings taken forty-eight hours after the beginning of barium. The auricular rate is quickened to 126 per minute. The ventricular rate is slightly increased over the prebarium level, reaching 30 per minute. Coupled ventricular extra systoles are also shown. Time intervals one-fifth and one-twenty-fifth seconds. Lower three strips represent a tracing taken after 220 mg. of barium chloride had been administered in ninety-six hours. The normal idioventricular rhythm is interrupted by frequent short runs of aberrant ventricular beats, occurring at a rate of approximately 210 per minute. The effective ventricular rate as a result is lowered. Time intervals one-fifth and one-twenty-fifth seconds.

lar in their occurrence and somewhat so in their shape. If one counts the ectopic beats, the average ventricular rate is considerably more rapid than before barium was begun. The number of effective ventricular contractions, however, was only 25 during this disturbance, whereas it was 35 before, and individual pauses between successive ventricular beats are quite as long as before barium was begun. This disturbance, near as it is to ventricular fibrillation, we regarded as an untoward effect of barium chloride and caused us to discontinue the drug.

A tracing, not presented, taken on the day after the drug was discontinued showed the same disturbance though the runs were not so long nor of such frequent occurrence. On the second day after the barium was stopped, no tracings were obtained but clinically isolated single ventricular extrasystoles were noted. The sudden death of the patient early in the morning of the third day after discontinuance of barium, prevented our determining how long the extrasystolic disturbance would continue after the barium had been stopped.

DISCUSSION

Lewis[6] has stated that one is justified in denoting runs of as many as six rapid ectopic ventricular beats as ventricular paroxysmal tachycardia. In our case runs of as many as seven ectopic beats were noted. The disturbance, therefore, can be called ventricular paroxysmal tachycardia or, at least, potential tachycardia. We do not believe that the sudden death of the patient was the result of this disturbance as the latter was definitely waning. We feel, however, that the occurrence of this disturbance on a relatively small dosage of barium chloride is worth a warning against giving the drug to the point of causing this potentially serious disturbance.

The disturbance in ventricular rhythm resulted from a dose of only 20 mg. three or four times a day for four days—a total dosage of only 220 mg. over a period of four days. This dosage is small, as the drug has been given by some of those who have reported upon it. Herrmann and Ashman used a dosage as high as 50 mg. every four hours for six doses per day for one week, without its having caused any serious disturbance in ventricular rhythm. The results that we have noted, as well as those of Strouss and Myer, make us feel that barium chloride is not a drug to be given in even moderate amounts without careful observation.

In spite of the increased irritability of the ventricle that resulted from barium, there still continue to be long pauses between successive ventricular beats, and the actual effective ventricular rate was slower. In other words, in our case barium induced a potentially serious disturbance in rhythm without removing the threat of ventricular standstill, or improving the circulation.

Elimination of barium is apparently rather slow. In our case isolated extrasystoles continued to be noted clinically forty-eight hours after the drug was discontinued.

CONCLUSIONS

A case is reported which during six years of observation had four attacks of complete A-V heart-block, lasting for varying lengths of time up to two months. During the third attack barium chloride may have been a factor in

restoring the sinus rhythm and preventing the onset of complete block again for eight months.

During the fourth attack of complete block which lasted four months and ended in death, barium chloride failed to increase the effective ventricular rate in doses of 20 mg. four times a day for four days.

This dosage of the drug brought on a marked extrasystolic disturbance with frequent short runs of rapid ventricular tachycardia. The same disturbance in the case of Strouss and Myer[5] is referred to.

The disturbance is reported because we regard it as potentially serious and an untoward result of barium chloride, and because the drug previously has been regarded as harmless in much larger doses than were required to bring about the disturbance in this and the other reported case.

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REGARDING THE EFFECT OF EPINEPHRIN UPON THE T-WAVE OF THE ELECTROCARDIOGRAM

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THE action of epinephrin upon the form of the T-wave of the axial electrocardiogram in the dog is constant when the vagi nerves are sectioned. The first effect is a negative influence upon the T-wave which occupies the early part of the reaction, usually before the greatest increase in the rate has occurred. It passes rapidly into the second stage of the effect, a positive influence upon the T-wave, which persists during the time the heart rate is increased. (Fig. 1.) An after effect of the action of epinephrin is often present, reappearance of a negative influence upon the T-wave, which then slowly returns to its original form. The primary negative influence upon the T-wave persists when the drug is injected directly into any of the cardiac chambers. If, however, the drug is injected in a manner which directs its distribution to the peripheral circulation before it arrives at the heart, i.e., into the aorta, the first or negative effect upon the T-wave is not present, and the T-wave changes begin with the positive influence.[1] (Fig. 2.)

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Received for publication, August 20, 1928.

[1]The experiments were upon 5 dogs under chloretone narcosis, artificial respiration, vagus nerve section, and opened thorax. The epinephrin was uniformly introduced into the blood stream in doses of 0.1 c.c. per 10 kilograms and diluted to 2 c.c. with saline solution. Electrocardiograms, using the axial lead (RA—LL), were taken at frequent intervals during the reaction. The total trials of the drug numbered twenty.

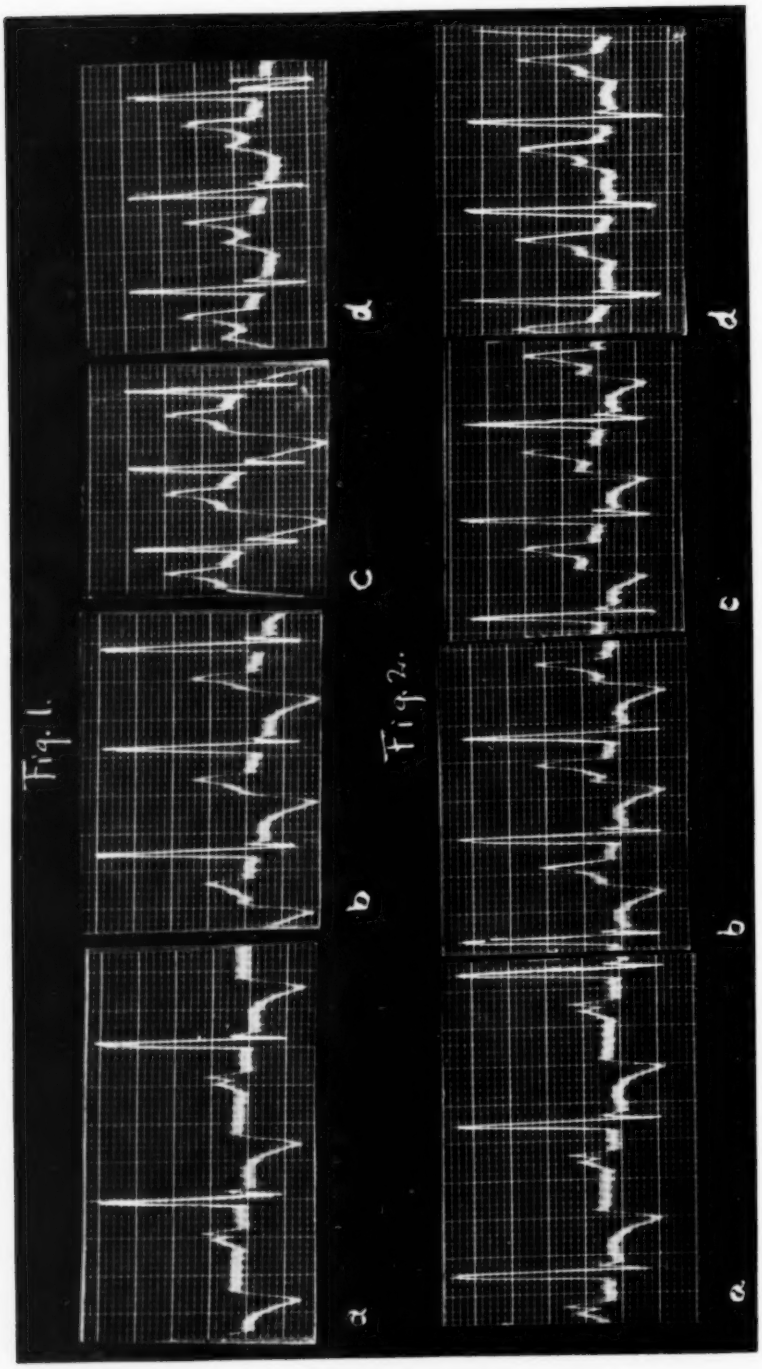

Fig. 1.—Axial (RA—LL) electrocardiogram. Time in fiftieth seconds. *a*. The normal curve. *b*, *c*, *d*, *e*. Successive electrocardiograms of the tachycardia following the administration of epinephrin into the superior vena cava.

Fig. 2.—The same animal. *a*. The normal curve. *b*, *c*, *d*. Electrocardiograms of the tachycardia following the administration of the epinephrin by way of the arch of the aorta.

Since the effect of the adrenalin is the same whether the pulmonary circuit is first exposed to the drug or this is avoided by injecting it into one of the left cardiac chambers, the initial negativity of the T-wave cannot be due to the action of the drug upon the endocardial lining of the right ventricle, or its Purkinje tissue nor to any action it may have upon the pulmonary capillaries or lung tissues (i.e., to a changed pressure in the pulmonary circuit). Since this preliminary influence upon the T-wave does not occur when the injection is made into the aorta, it is in all probability related to the earlier entry of the adrenalin into the coronary circulation, and therefore the negative influence of the epinephrin upon the T-wave of the axial electrocardiogram is a direct cardiac effect of the drug whereas the positive influence is an effect which is indirectly produced by its peripheral action. This does not, however, suggest that epinephrin has a direct action upon the heart distinct from that which occurs through the accelerator nerve endings. The absence of the initial effect upon the T-wave is simply due to the sudden increase of the blood pressure following the intense constriction at the periphery, which tends to elevate the T-wave and mask the reverse effect.

LABORATORY METHODS

A STUDY OF THE NATURE OF THE UREA CONCENTRATION TEST AND ITS VALUE AS A TEST OF RENAL FUNCTION*

By M. M. Wintrobe, B.A., M.D., B.Sc. (Med.), New Orleans, La.

In 1920 MacLean and de Wesselow[1] introduced a simple test of renal function based on the response of the kidneys to the ingestion of a definite quantity of urea. The concentration of urea in two urine specimens, collected at hourly intervals after the taking of urea, is determined by a chemical method requiring only two minutes in its performance, and of such a simple nature that the method is as readily available to the country practitioner as for the internist with elaborate laboratory facilities at his disposal.

Besides its simplicity many theoretical advantages may be adduced in favor of such a test as an indicator of renal function. Urea is a normal end-product of protein metabolism, and its excretion by the kidney is one of the important functions of that organ. Following administration, urea undergoes no chemical change and normally its rate of excretion is rapid and is governed by its concentration in the blood.[2] Conceivably the rate of absorption of urea from the alimentary tract may be delayed. However, Harrison[3] found normal results in 7 cases of gastrointestinal disease. Moreover, in cases of impaired circulation, as from heart failure, absorption from the alimentary tract is undoubtedly better than from the periphery. A great deal of work must be done by the kidney in eliminating urea and, as Rabinowitch[4] points out, the greater the degree of concentration, the greater the work per gram. By the ingestion of urea, then, the actual capacity of the kidney to do additional work is measured.

For the estimation of urea, MacLean[5] describes a simple apparatus made with a graduated burette, a bottle, a bell-shaped vessel, and some rubber tubing. Sodium hypobromite is used for the liberation of nitrogen from the urine, and a table showing the percentage of urea equivalent to cubic centimeters of nitrogen evolved, is given. This apparatus I have found to be more accurate and easier to manipulate than the more commonly used Doremus ureometer.

The hypobromite method of estimating urea, although not perfectly accurate, is nevertheless exact enough for ordinary clinical work. Harrison,[3] investigating the effect of proteinuria on the urea concentration reading, found that as much as 2 per cent of albumin made no appreciable difference. Ordinary degrees of hematuria, also, had no influence on the test. Harrison gives a simple method of removing protein from the urine, which can be used when large quantities of albumin are present.

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Received for publication, August 24, 1928.

MacLean[5] records excellent results with the urea concentration test in a series of more than 10,000 cases. Rabinowitch[2] likewise reports good results. Bowen,[6] in a comparative study of various renal function tests, concluded that the urea test was more delicate than the phenolsulphonephthalein test and found it corresponding better with the day-night urine volume ratio and specific gravity, which Christian and O'Hare[7] consider the most delicate test of all, than did the phenolsulphonephthalein test or blood urea nitrogen. In spite of these favorable reports the test has not been generally adopted in America. For this reason it has been thought worth while to determine the value of this test as compared, particularly, with the widely used phenolsulphonephthalein test. Some observations on the relation of these two tests have been reported briefly elsewhere.[8] The results of renal function tests in 49 healthy students and 56 hospital patients are here considered.

RENAL FUNCTION IN HEALTHY INDIVIDUALS

Methods.—All the students examined were in good health, and urinalysis was negative in each case. Tests were made in the afternoon one or more hours after a light lunch.

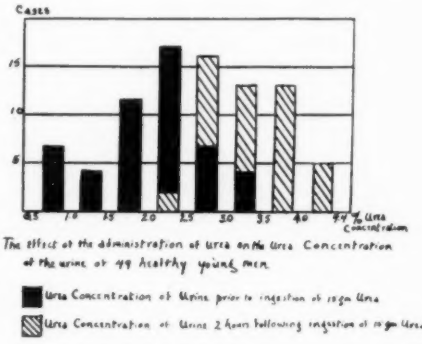

Fig. 1. The effect of the administration of urea on the Urea Concentration of the urine of 49 healthy young men.

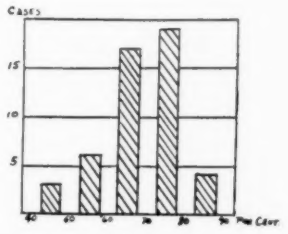

Fig. 2. Phthalein Excretion in 49 Healthy Young Men.

Urea Concentration Test.—Fluids were somewhat restricted for two hours prior to the giving of urea as well as during the test. In order to determine the concentration of urea in urine under normal conditions, the urea concentration of specimens voided just before the administration of urea was determined. At one hour and two hours following the ingestion of 15 gm. of urea, dissolved in 100 c.c. of water, urine was collected, its quantity measured, and the urea concentration determined by MacLean's technic.[5] Urine specimens of more than 120 c.c. were discarded.

Phenolsulphonephthalein Test.—A phenolsulphonephthalein test was carried out on each student within a few days of the time of performing the urea concentration test. The dye was injected intravenously in all cases and the colors matched without the aid of a colorimeter.

Forty-nine students were examined. Maximum urea concentration varied from 2.4 per cent to 4.4 per cent, the average being 3.22 per cent. In only 2 instances was the urea concentration slightly below (2.46 per cent and 2.4 per

cent) the generally accepted normal (2.5 per cent). Concentration of urea in the urine prior to the giving of urea varied from 0.6 per cent to 3.4 per cent with an average of 1.49 per cent. In Fig. 1 the frequency distribution of these findings is shown, and the effect of the administration of urea on the urea concentration of the urine indicated.

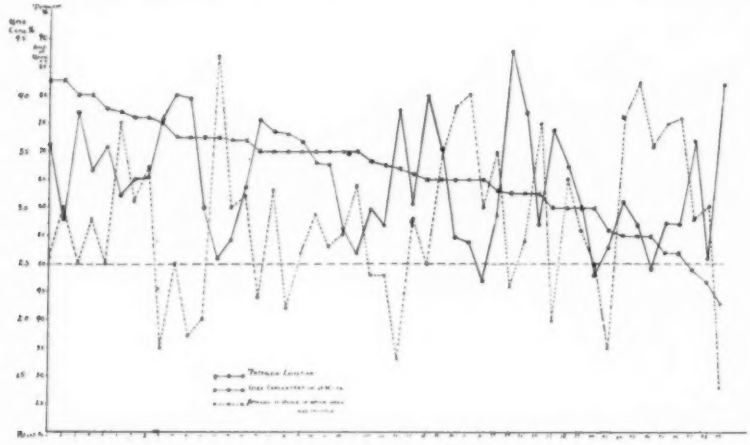

Chart I.—Relation of phthalein excretion to maximum urea concentration of urine following ingestion of 15 gm. of urea. Relation of amount of urine to urea concentration.

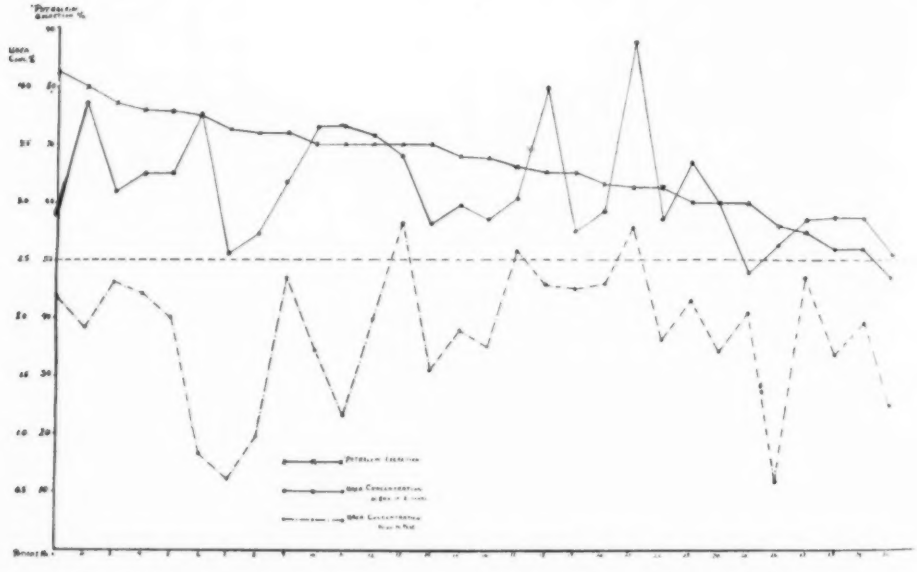

Chart II.—Relation of urea concentration two hours following ingestion of 15 gm. urea to urea concentration of urine prior to test.

Phenolsulphonephthalein excretion varied from 43 per cent to 82.5 per cent with an average of 66.3 per cent. Fig. 2 shows the frequency distribution of these findings. It will be seen that the phenolsulphonephthalein reading was below 50 per cent in 3 instances (49, 47, 43 per cent, respectively).

Chart I is an attempt to determine the value of the urea concentration test as a quantitative indicator of renal function. The results of all the phenolsulphonephthalein determinations have been arranged in descending order, and, in this order, the phenolsulphonephthalein percentage and corresponding maximum urea concentration in each case are plotted. It is apparent that the two curves do not coincide as they should if they were both quantitative indicators of the excretory power of the kidneys.

In the same chart, the amount of urine in which the concentration of urea was measured is plotted in relation to the corresponding urea concentration. It appears from this chart that quantity of urine has a definite relationship to concentration of urea, concentration being high when the amount of urine is low and vice versa. MacLean's instructions that urine samples of 120 c.c. or more should be discarded, recognize this influence of volume on concentration of urea. Judging by the variation apparent when even quantities of less than 120 c.c. are considered, one is led to doubt the value of the urea concentration test as a *quantitative* indicator of renal function. It must be remembered, however that such a conclusion is based on the hypothesis that phenolsulphonephthalein excretion indicates quantitatively the excretory power of the kidneys.

Another factor which influences the maximum urea concentration of urine following the ingestion of urea is the amount of urea normally being excreted by the individual. We have already seen that this amount varies a good deal from individual to individual. Some idea of the relationship of these two figures may be gained by determining the urea concentration in urine just prior to the ingestion of urea. In Chart II the normal urea concentration of the urine of 38 students is plotted in relation to the urea concentration two hours following the ingestion of 15 gm. of urea. The two curves appear to coincide, although somewhat irregularly.

To sum up, then, both the urea concentration and phenolsulphonephthalein tests were fairly consistent in indicating a healthy state of the kidneys in 49 healthy students. As quantitative indicators of the excretory power of the kidneys, however, the two tests did not agree. The maximum urea concentration reading appears to be related to the quantity of urine in which it is excreted and perhaps, also, to the amount of urea normally being excreted by the individual.

COMPARISON OF RENAL FUNCTION TESTS IN 56 PATIENTS

In order to determine the relative value of the urea concentration test in the detection of disease, as well as to discover the importance of the observations just noted, 56 clinic and ward patients suspected of having some form of kidney disease were examined. Urinalysis, phenolsulphonephthalein, and at least one urea concentration test were carried out on each, and blood chemistry determinations were made on the majority.

The *phenolsulphonephthalein* test was carried out as in the case of the students, but the dye was injected intramuscularly. A total excretion of 40 per cent of the dye or less was considered definitely abnormal, while determinations ranging between this figure and 50 per cent were considered borderline.

The *urea concentration* test was carried out as already described. The tests were made in the morning and the patients were allowed no food and little fluid after the preceding evening meal.

Blood determinations were made in the routine manner. Nonprotein nitrogen was considered normal up to 35 mg., urea nitrogen up to 15 mg., creatinine, 2.5 mg., and uric acid, 4.5 mg. per 100 c.c. of blood.

Results of the examination of these 56 patients are given in detail in Table I and summarized in Table II.

ANALYSIS OF RESULTS

Urinalysis.—Albumin or casts in the urine indicated the possible presence of renal disease in 60 per cent of the cases.

Urea Concentration and Phenolsulphonephthalein Tests.—Concentration of urea was below normal in 60 per cent of the cases, while phenolsulphonephthalein was 40 per cent or less in 62.5 per cent of the cases and 45 per cent in 12.5 per cent. Of 17 cases of early or subacute nephritis (Table I, Groups 2 (b), and 4) the urea concentration test indicated the presence of disease in 16, while phenolsulphonephthalein excretion was abnormal in only 10. In other words, phenolsulphonephthalein excretion was normal in 7 patients who had other evidence of renal disease.

On the other hand, of 20 patients with predominating symptoms and signs of heart disease (Table I, Groups 2 (c), 2 (e), and 3) phenolsulphonephthalein excretion was 40 per cent or less in 15 and 45 per cent in 3 more, while urea concentration was below normal in only 9 individuals. Eleven patients with pronounced cardiac symptoms and much edema, but with little or no renal impairment, as indicated by normal urea concentration and negative urine and blood findings, showed a phenolsulphonephthalein excretion of 45 per cent or less. It appears that in many cases of cardiac disease with edema phenolsulphonephthalein excretion is a poor test of renal function, as in such cases the absorption of the dye by the peripheral circulation is greatly impaired. This is in harmony with the experience of Christian and O'Hare,[7] who state that in cardiac passive congestion phenolsulphonephthalein excretion may be moderately low without any real kidney disease.

Nonprotein Nitrogen and Urea Nitrogen.—In only 23 per cent of the patients was there even slight abnormality of nonprotein nitrogen, while urea nitrogen was above normal in 43 per cent. This is consistent with the statement of other observers that an increase of the chemical constituents of the blood is a late sign of renal disease.

Creatinine and Uric Acid.—Creatinine was above normal in only 1 patient, while uric acid was above 4.5 mg. in 16 cases; 3 of these showed no other evidence of renal disease, while 4 had definite nephritis with normal nonprotein nitrogen and urea nitrogen.

Returning to the question of the value of the urea concentration test as a quantitative indicator of renal function, it may be said that calculations devised to allow for the relation of amount of urine voided (Chart I) and concentration of urea prior to the test (Chart II)) to maximum urea concentration have

TABLE I

RENAL FUNCTION TESTS IN PATIENTS

NO.	AGE	BLOOD PRESSURE	URINALYSIS	BLOOD UREA NITROGEN MG.*	PHTHALEIN PER CENT 130 MIN.	UREA CONCENTRATION OF URINE						REMARKS
						BEFORE TEST		AFTER TAKING 15 GM. UREA				
								1 HR. AFTER		2 HR. AFTER		
						AMT. URINE C.C.	UREA PER CENT	AMT. URINE C.C.	UREA PER CENT	AMT. URINE C.C.	UREA PER CENT	
1. Patients Showing no Evidence of Renal Disease												
1	21	124/ 84	0	12	50			98	2.24	66	3.07	Syphilis. Renal function tested
2	20	120/ 80	0	15	50			90	1.18	55	2.52	Tonsillitis. Nocturia
3	13		0	15	80			80	1.97	65	2.80	
4	20	119/ 80	0	15	45	42	2.20	96	2.31	35	3.45	Recovered from acute nephritis
5	12	98/ 46	0	14	45	70	0.60	44	3.31	46	3.60	Constipation. Syphilis. Headache
6	20	120/ 92	0	14	40	60	1.66	86	1.88	27	3.39	Tonsillitis
						60	0.65	85	2.32	95	2.97	
								22	1.45	61	3.44	Headaches
2. Hypertension (a) With no Laboratory Evidence of Nephritis and no Signs of Heart Disease												
7	61	210/162	0	15	60	56	2.51	69	3.02	66	3.32	Nocturia ++
8	49	226/120	0	14	85	23	1.19	78	2.44	28	3.00	G. I. Ringing in L. ear
9	37	186/ 84	0	13	80	10	1.95	60	3.04	74	3.45	Nocturia ++. Slight palp. & dysp.
10	42	175/115	0	15	50			48	3.44	40	3.75	Focal infection. Nocturia
11	37	160/100	0	17	47	19	1.38	75	2.31	59	3.15	Headache. Nocturia +

*Where other constituents of the blood are abnormal they are recorded under "Remarks."

TABLE I—CONT'D

NO.	AGE	BLOOD PRESSURE	URINALYSIS	BLOOD UREA NITROGEN MG.*	PHTHALEIN PER CENT 130 MIN.	UREA CONCENTRATION OF URINE						REMARKS
						BEFORE TEST		AFTER TAKING 15 GM. UREA				
								1 HR. AFTER		2 HR. AFTER		
						AMT. URINE C.C.	UREA PER CENT	AMT. URINE C.C.	UREA PER CENT	AMT. URINE C.C.	UREA PER CENT	
2. Hypertension (b) With Beginning Signs of Nephritis but Few or no Cardiac Symptoms												
12	39	192/102	Hy. casts +	15	80	81	0.33	69	2.41	55	2.26	Nocturia ++. Slight dysp. Uric acid 6 mg.
13	52	210/110	Alb. + Hy. C. +	15	40			85	0.90	62	1.41	Neuralgia. Nocturia +++
14	51	222/148	Alb. + Hy. & G.C. +	16	25					40	1.74	Sl. dysp. Nocturia ++. Uric acid 7.5 mg.
15	32	168/ 90	Alb. +	12	30			90	1.21	79	2.37	Syphilis. G. I. Uric acid 6 mg.
16	32	164/ 84	0	15	50			110	1.01	75	2.17	Focal inf. (tonsils)
17	39	200/120	Hy. C. ++ G.C. +	15	30	8	1.15	18	2.71	46	2.60	Headaches
						12	1.13	48	2.38	38	2.60	
18	29	154/ 90	0	14	60	100	0.74	94	1.31	91	1.63	Nocturia +. Occ. headaches and dizzy spells
						91	0.45	31	1.16	100	1.63	
						45	0.86	93	1.54	90	1.58	
19	52	174/ 94	0	14	70	30	0.74	54	1.29	72	1.41	Slight dysp. Dizziness. Headaches
						74	0.61	68	1.35	76	1.69	
2. Hypertension (c) With Beginning Cardiac Disease, Little or no Nephritis												
20	42	275/140	Alb. +	20	45	100	2.78	53	2.57	64	2.65	Slight dysp. Palp. Edema. N. P. N. 40 mg. Uric acid 6.6 mg.
21	38	210/110	0	15	40	53	1.70	57	2.63	77	2.48	Syphilitic heart disease
						55	2.04	50	2.61	72	2.64	
22	46	270/170	Hy. C. +	24	20	53	0.95	23	2.00	9	2.47	Precordial pain. Dysp. Palp. Cough. N. P. N. 48 mg.
					35							
23	58	148/112	0	27	35	36	1.17	34	2.34	33	2.54	Dysp. and ectopics. N. P. N. 45 mg. Uric acid 6 mg.
											1.41	
24	63	240/134	Pus +	22	45	10	0.86	63	1.25	100		Edema and sl. dysp.

TABLE I—CONT'D

NO.	AGE	BLOOD PRESSURE	URINALYSIS	BLOOD UREA NITROGEN MG.*	PHTHALEIN PER CENT 130 MIN.	UREA CONCENTRATION OF URINE						REMARKS
						BEFORE TEST		AFTER TAKING 15 GM. UREA				
								1 HR. AFTER		2 HR. AFTER		
						AMT. URINE C.C.	UREA PER CENT	AMT. URINE C.C.	UREA PER CENT	AMT. URINE C.C.	UREA PER CENT	
2. Hypertension (d) With Well Marked Nephritis, Few or no Cardiac Symptoms												
25	52	204/108	Alb. ++ Hy. & G.C. ++ R.B.C. +	60	10			120	1.51	112	1.76	Autopsy: primary kidney disease. Died in uremia. N. P. N. 100 mg. Uric acid 7 mg.
26	45	220/160	Alb. ++++ G.C. + R.B.C. +	25	30	230	0.78	65	1.06	40	1.41	Advanced chr. neph. N. P. N. 44 mg. Uric acid 8 mg.
27	33	260/150	Hy. & G.C. + R.B.C. + Pus +	38	15			81	1.35	103	1.22	Nausea. Edema. Nocturia ++. N. P. N. 75 mg. Uric acid 6 mg.
28	62	170/100	Alb. ++ Hy. & G.C. + R.B.C. + Pus +	15	35			96	1.31	8	1.91	Pellagra
29	48	180/ 90	Alb. ++ Pus +		20			120	0.64	110	0.73	Dizziness. Blinding spells. Nocturia ++
30	41	190/ 98	Alb. ++ G.C. +	21	10	44	0.72	73	0.97	65	0.85	Vertigo. Dimness of vision. Nocturia ++ N. P. N. 40 mg.
31	36	198/138	Alb. +++ Hy. & G.C. + R.B.C. + Pus +	45	10	92	0.58	24	0.78	39	0.79	Subacute neph. with edema. N. P. N. 80 mg. Uric acid 10 mg.
32	44	190/130	Alb. +++ Hy. & G. C. ++ R.B.C. + Pus ++	20	40					100	1.42	Chr. neph. Heart failure controlled by digitalis. N. P. N. 40 mg. Uric acid 5 mg.

TABLE I—CONT'D

NO.	AGE	BLOOD PRESSURE	URINALYSIS	BLOOD UREA NITROGEN MG.*	PHTHALEIN PER CENT 130 MIN.	UREA CONCENTRATION OF URINE						REMARKS
						BEFORE TEST		AFTER TAKING 15 GM. UREA				
								1 HR. AFTER		2 HR. AFTER		
						AMT. URINE C.C.	UREA PER CENT	AMT. URINE C.C.	UREA PER CENT	AMT. URINE C.C.	UREA PER CENT	
2. Hypertension (e) With Marked Heart Failure Predominating												
33	52	220/120	Alb. +		50			75	1.43	97	1.93	Fibrillation. Heart failure
34	45	242/148	Alb. ++ Hy. C. +	15	30			52	1.69	54	2.16	Marked heart failure. Uric acid 6 mg.
35	48	160/ 80	Hlb. ++ Hy. & G.C. + R.B.C. + Pus ++	17	22			51	2.63	50	3.75	Heart failure
36	59	190/140	Alb. ++		40			98	1.29	90	1.79	Marked heart failure
37	36	200/ 45	0		25			83	2.41	54	2.6	Aortic regurgitation with failure
38	45	208/150	0		20			98	1.66	85	1.99	Syphilitic heart disease with failure
39	52	160/ 58	Alb. ++ Hy. & G.C. + Pus ++	18	15			91	1.88	65	1.82	Heart failure. Uric acid 6 mg.
40	70	170/110	Alb. + G.C. +	19	15	115	0.97	100	1.02	104	1.10	Marked heart failure. N. P. N. 38 mg.
41	45	160/ 80	Pus +	15	20			92	0.93	99	1.04	Heart failure
42	60	165/ 90	Alb. + Hy. & G.C. + Pus +		20			85	2.27	70	2.33	Syphilitic heart and vascular disease
43	49	155/115	Pus +++		38			50	3.00	66	2.98	Irregular and failing heart. Nocturia +++
44	74	202/140	0	17	35	172	2.09	68	2.52	39	2.75	Heart failure
45	32	170/120	Alb. ++ G.C. + Pus +	15	25	80	2.92	34	3.14			Heart failure. Uric acid 6 mg.

TABLE I—CONT'D

NO.	AGE	BLOOD PRESSURE	URINALYSIS	BLOOD UREA NITROGEN MG.*	PHTHALEIN PER CENT 130 MIN.	UREA CONCENTRATION OF URINE						REMARKS
						BEFORE TEST		AFTER TAKING 15 GM. UREA				
								1 HR. AFTER		2 HR. AFTER		
						AMT. URINE C.C.	UREA PER CENT	AMT. URINE C.C.	UREA PER CENT	AMT. URINE C.C.	UREA PER CENT	
3. Heart Failure, no Hypertension and no (?) Nephritis												
46	24	138/114	0	15	40	192	1.55	60	2.61	36	3.39	Acute heart failure. Uric acid 5.5 mg.
47	36	124/ 88	Alb. +	15	45	16 16	2.46 1.48	62 73	3.55 2.84	67 41	3.62 3.69	Aortic and myocardial heart disease. Sl. failure. Uric acid 5.5 mg.
4. Subacute Nephritis With no Hypertension or Heart Disease												
48	24	150/100	Alb. +++ Hy. C. +	90	5	105	0.54	51	0.66	36	0.67	Followed exposure to cold. N. P. N. 150 mg. Creatinine 3.5 mg. Uric acid 10 mg.
49	20	120/ 80	Alb. ++ Hy. & G.C. + R.B.C. ++++	16	65	60	1.38	102	1.64	85	2.37	Followed streptococcal tonsillitis
50	18	130/ 95	Alb. + Hy. C. +	14	20	70	0.4	91	1.07	60	1.27	Followed typhoid vaccination
51	37	135/ 95	Alb. ++++ Hy. & G.C. ++ R.B.C. +	17	45			96	1.81	66	2.19	Cause ? Uric acid 5.5 mg.
52	38	140/ 94	Alb. ++ Hy. & G. C. + R.B.C. +	17	30	85	1.53	22	1.88	52	2.09	Uric acid 5.5 mg.
53	13	110/ 70	Alb. ++	15	30			77	1.69	75	2.42	Followed tonsillitis
54	41	102/ 80	G.C. +	14	40			56	1.85	54	2.31	Mercurial
55	54	125/ 70	Alb. + Hy. C. + R.B.C. & Pus ++		35			36	1.51	44	2.08	Bronchiectasis
56	44	112/ 68	Hy. & G. C. +	19	60	38	1.78	25	2.34	64	2.10	Focal nephritis

TABLE II

SUMMARY OF RENAL FUNCTION TESTS IN 56 PATIENTS

CASES			BLOOD				PHTHALEIN EXCRETION		MAXIMUM	
TYPE	NO.	URINALYSIS SHOWED ABNORMALITY IN	NONPROTEIN NITROGEN ABOVE 35 MG. IN	UREA NITROGEN ABOVE 15 MG. IN	CREATININE ABOVE 2.5 MG. IN	URIC ACID ABOVE 4.5 MG. IN	40 PER CENT OR LESS IN	ABOVE 40 PER CENT BELOW 50 PER CENT IN	UREA CONCENTRATION BELOW 2.5 PER CENT IN	SYMPTOMS SUGGESTIVE OF NEPHRITIS IN
1. No evidence of renal disease	6	0	0	0	0	0	1	2	0	2
2. Hypertension										
(a) with no nephritis	5	0	0	1	0	0	0	1	0	4
(b) with beginning signs of nephritis but few or no cardiac symptoms	8	5	0	1	0	3	4	0	7	5
(c) with beginning cardiac disease, little or no nephritis	5	2	4	4	0	2	3	2	1	0
(d) with well marked nephritis, few or no cardiac symptoms	8	8	6	6	0	5	8	0	8	8
(e) with marked heart failure predominating	13	9	1	4	0	3	12	0	8	
3. Heart failure no hypertension, no (?) nephritis	2	1	0	0	0	2	1	1	0	1?
4. Subacute nephritis no hypertension or heart disease	9	9	2	5	1	3	6	1	9	9
Total	56	34	13	21	1	18	35	7	33	29

failed to improve the significance of the test. The most obvious solution to the hypothetical failure of the urea concentration test as a quantitative indicator of renal function, considered in the discussion of Charts I and II, is the calculation of the total amount of urea excreted in two hours. This was done in 49 students and found to range from 2.2 gm. to 5.2 gm. with an average of 3.9 gm. Patients suffering from various grades of nephritis excreted from 0.5 gm. to 3.8 gm. In cases of severe renal damage, total urea excretion was very low, but in moderate degrees of nephritis total urea excretion often greatly exceeded the lower limits of normal observed in students. It appears, then, that it is the concentrating power of the kidney, rather than the amount of urea excreted, that is first affected in nephritis.

A true conception of the value of the urea concentration test as a quantitative indicator of renal function can only be gained by following the progress of a large number of cases with repeated tests. As this was done in only a relatively few cases, no more can here be said than that the urea concentration test showed some relation to the degree of renal impairment. Jones and Cantarow[9] suggest that the percentage variation between urea concentration prior to the test and urea concentration following ingestion of urea is more significant of renal damage than maximum urea concentration alone. Judging by the data for the 56 patients here reported, however, little additional information is to be gained by the detailed calculation of percentage variation.

Conclusions.—Power of concentration appears to be a definite function of the kidney and one which is early involved in the damage produced by nephritis. Thus, most observers agree that the earliest sign of kidney damage as shown by laboratory tests is an increase of the volume of the night urine and a decrease in its specific gravity, or, in other words, a decrease in its concentration. MacLean's test measures the concentrating power of the kidney in response to a definite stimulus by a method which is even more simple than that necessary for the carrying out of the various water concentration tests, and in both theory and practice is a valuable test of renal function. Where there is congestive cardiac failure, the urea concentration test probably gives a more correct conception of the functional state of the kidneys than does the phenolsulphonephthalein test. As Rabinowitch points out[10] no single test gives a complete and uniform idea of renal function, and various types of nephritis show varied responses to each test. The urea concentration test is as important in the detection of renal disease as is the widely-used phenolsulphonephthalein test, and because of its simplicity is particularly valuable for the physician with few laboratory facilities.

SUMMARY

1. Urea concentration and phenolsulphonephthalein tests in 49 healthy individuals are compared, and the value of these tests, as well as of urinalyses and blood chemistry determinations, is considered in 56 patients.

2. The nature of the urea concentration test and the relation of maximum urea concentration to total urine volume and to urea concentration of urine prior to the ingestion of urea are examined.

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AMINO NITROGEN DETERMINATION IN BACTERIOLOGIC MEDIA*

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IN RECENT years the bacteriologist has resorted to minute biochemical analyses in an attempt to facilitate the differentiation of closely allied strains. The determination of amino nitrogen has become a common procedure in the study of protein metabolism by the bacteria.

Sörensen's formol titration,[1] Van Slyke's nitrous acid method,[2] and the newer colorimetric method of Folin[3] have all been employed. Because of its speed and reported accuracy the van Slyke method has been most frequently chosen recently.

Several investigators have reported unsatisfactory results with the Van Slyke apparatus as applied to bacteriologic culture media. The difficulty has been in obtaining consistent results on duplicate samples of the same media.

As has been pointed out by Lamson,[4] Sturges and Rettger,[5] and Wagner, Dozier, and Meyer,[6] found it necessary to make several determinations by this method before satisfactory checks could be obtained. Lamson[4] concluded that Van Slyke's method yields very irregular results, and is unsatisfactory for solutions low in amino nitrogen. De Bord[7] abandoned the method upon finding as much as 18 per cent variation in a series of tests on sterile peptone solution.

In the course of an investigation on the enzymes of the streptococci by one of us, the Van Slyke method was adopted for the determination of protein cleavage. Despite great care in attention to all details of the procedure, satisfactory checks could not be obtained. One example will suffice to illustrate the difficulty.

The medium tested was sterile veal infusion broth adjusted to a P_H 7.6. The Van Slyke micro apparatus was employed, using a 2 c.c. sample and Pfanstiehl caprylic alcohol for the prevention of foaming. Table I indicates

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Received for publication, August 11, 1928.

†Aided by the Rockefeller Grant for Research.

the results in one experiment. The amount of gas from the blank on the reagents has been deducted. Temperature and pressure remained constant. Ammonia was not removed.

The results in Table I, representative of many other such experiments, indicate the inconsistency we experienced with this method. A maximum difference of 44.9 per cent between checks made it quite obvious that the method could not be used. It seemed to us that with a method commonly relied upon by bacteriologists it would be worth while to determine the reason for such inconstancy. We desire, therefore, to report our findings regarding the reasons for the inconstant results by the Van Slyke method, and a comparison of this method with those of Sörensen and Folin as applied to bacteriologic media.

TABLE I

VAN SLYKE NITROGEN ($NH_2 + NH_3$) IN STERILE VEAL INFUSION BROTH

SAMPLE	GAS IN C.C.	AMINO N ($+NH_2$) MG. PER 100 C.C.	PER CENT DEVIATION FROM THE MEAN
1	1.87	50.53	+26.3
2	1.03	28.00	−30.0
3	1.39	37.78	− 5.5
4	1.21	32.89	−17.7
5	1.68	50.83	+27.0
Mean	1.44	40.00	

Upon examination of the literature we found a reference to the importance of the caprylic alcohol foam depressant in a paper by Bock.[8] In a footnote he calls attention to the necessity for purifying the caprylic alcohol.

An experiment was undertaken to determine the relation of the caprylic alcohol to the discrepancy in our results. Some of the Pfanstiehl caprylic alcohol, which we had been using, was purified according to the method of Bock. Since this process of purification has not been brought sufficiently to the attention of bacteriologists we quote the procedure here. "The alcohol is shaken twice (best in a separatory funnel) with a mixture of glacial acetic acid and $NaNO_2$ solution (30 gm. in 100 c.c. H_2O), the acid and the nitrate being in the proportion of 1:5." "The alcohol is then washed with a little water two or three times, transferred to a distilling flask, a very small fraction of NaOH added, and distilled under reduced pressure."

Table II shows the results of determinations with this purified Pfanstiehl caprylic alcohol as compared with the unpurified Pfanstiehl and several other brands. The determinations were made using the regular reagents, 2 c.c.

TABLE II

VOLUME OF GAS PER C.C. CAPRYLIC ALCOHOL, 0° C., 760 MM. PRESSURE

PURIFIED		UNPURIFIED			
SAMPLE	PFANSTIEHL	PFANSTIEHL	MERCK'S	COLEMAN BELL	EASTMAN
1	0.1615 c.c.	4.69 c.c.	0.200 c.c.	0.104 c.c.	0.630 c.c.
2	0.175 c.c.	4.44 c.c.	0.256 c.c.	0.076 c.c.	0.596 c.c.
3	0.166 c.c.	4.52 c.c.	0.220 c.c.	0.114 c.c.	0.622 c.c.
4			0.230 c.c.	0.084 c.c.	0.604 c.c.
5				0.090 c.c.	
Average	0.1677 c.c.	4.55 c.c.	0.227 c.c.	0.094 c.c.	0.613 c.c.

distilled water as the sample, and 0.5 c.c. of the caprylic alcohol in each case. The figures indicate the volume of gas derived per c.c. of alcohol, calculated to 0° C. and 760 mm. pressure.

From Table II it is evident that there is a wide variation in the purity of commercial caprylic alcohols. The sample used should be purified if preliminary tests indicate appreciable variation or large amounts of gas.

The Bock[8] method of purification yields an alcohol which gives consistently satisfactory checks on blank determinations. It is further evident that in the Van Slyke procedure the amount of caprylic alcohol must be accurately measured.

The effect of the shaking period on the volume of gas obtained from the purified caprylic alcohol was next determined. The usual reagents were used plus 2 c.c. of water as the test material, with and without the purified alcohol. Three minutes were allowed for absorption, and the shaking interval was varied. Table III indicates the results.

TABLE III

EFFECT OF SHAKING TIME ON VOLUME OF GAS IN BLANK DETERMINATIONS

SAMPLE	REAGENTS PLUS 2 C.C. H_2O; NO ALCOHOL	REAGENTS PLUS 2 C.C. H_2O; 0.2 C.C. PURIFIED ALCOHOL	
	SHAKEN 5 MIN.	SHAKEN 5 MIN.	SHAKEN 10 MIN.
1	0.09 c.c.	0.27 c.c.	0.41 c.c.
2	0.11 c.c.	0.30 c.c.	0.40 c.c.
3	0.13 c.c.	0.26 c.c.	0.39 c.c.
4	0.13 c.c.		
5	0.10 c.c.		
6	0.11 c.c.		
Average	0.11 c.c.	0.28 c.c.	0.40 c.c.

From the above experiment it can be seen that a definite shaking period must be used in the test, and that it should be the same as that employed in the blank on the reagents. It is also apparent that even the purified caprylic alcohol adds an important amount of gas to the total volume.

Having established the fact that satisfactory checks could be obtained with the purified alcohol on blank determinations, we again applied the method to veal infusion broth. Table IV shows the results obtained on two different lots of sterile veal infusion broth adjusted to P_H 7.6. Two c.c. of the

TABLE IV

VAN SLYKE AMINO NITROGEN IN STERILE VEAL INFUSION BROTH

SAMPLE	BROTH NO. 1		BROTH NO. 2	
	MG. PER 100 C.C.	PER CENT DEVIATION FROM MEAN	MG. PER 100 C.C.	PER CENT DEVIATION FROM MEAN
1	36.55	−7.7	39.20	+0.9
2	38.50	−2.7	38.85	0.0
3	41.00	+3.5	38.20	−1.6
4	40.50	+2.2	38.45	−1.0
5	41.50	+4.7	39.35	+1.3
6			38.05	−2.0
7			39.50	+1.6
8			39.20	+0.9
Mean	39.60		38.85	

test sample and 0.2 c.c. purified caprylic alcohol were used, allowing a five-minute shaking period followed by three minutes absorption. The free NH_3 was determined by the Folin[9] aeration method, and was found to be 10 mg. per 100 c.c. of broth. This amount has been deducted from the results in Table IV.

The data in Table IV represent the unselected results in successive determinations. It can be seen that these results are fairly consistent, and within the experimental error in measuring such small quantities of gas.

A COMPARATIVE STUDY OF THE VAN SLYKE AND FOLIN METHODS FOR THE DETERMINATION OF THE AMINO NITROGEN IN CULTURE MEDIA

While the data reported above satisfied us that the Van Slyke method would give satisfactory results with certain precautions, we thought it advisable to compare this method with the newer colorimetric one of Folin.[3]

Two samples of sterile 1 per cent Difco peptone water were examined by these two methods. One was unadjusted, and the other buffered with phosphates to a P_H 7.6. Table VI indicates the results obtained. Ammonia was not removed, since Nessler's reagent failed to show any NH_3 present.

TABLE VI

AMINO NITROGEN (PLUS NH_3-N) IN 1 PER CENT PEPTONE WATER BY VAN SLYKE'S AND FOLIN'S METHODS

SAMPLE	MG. PER 100 C.C.			
	UNBUFFERED		BUFFERED	
	VAN SLYKE	FOLIN	VAN SLYKE	FOLIN
1	6.45	4.74	5.40	4.63
2	5.95	4.78	5.50	4.74
3	5.65	4.65	5.20	4.69
4			5.40	
5			5.20	
Mean	6.01	4.72	5.35	4.69

An average difference between the methods of 21.4 per cent for the unbuffered and 12.6 per cent for the buffered peptone water, was larger than we anticipated. The lack of agreement may be explained by the difficulty in measuring such small quantities of gas as are obtained from 2 c.c. samples of a medium low in amino nitrogen.

In order to check the accuracy of the methods a definite known amount of amino acid, giving a larger volume of gas, was added to the peptone water media. Standard glycine solutions were prepared by adding 2.5 mg. of nitrogen in the form of glycine to 50 c.c. portions of the buffered and unbuffered peptone water. Using the value 4.72 mg. per 100 c.c. peptone water and 4.69 mg. per 100 c.c. unbuffered peptone water from Table VI as the amino nitrogen content of the respective media, the addition of 5 c.c. of standard glycine solution (representing 5 mg. amino N.) to 100 c.c. of the media would give a calculated amino nitrogen content of 9.25 mg. and 9.23 mg. respectively. The amount of amino nitrogen actually determined experimentally by both methods is indicated in Table VII. Nessler's reagent showed no NH_3 present.

With the added amount of amino nitrogen the results are very acceptable particularly by the Van Slyke method. This would indicate that the error with the Van Slyke method in Table VI is largely due to the small amount of gas measured.

The two methods were now applied to the determination of amino nitrogen in two lots of veal infusion broth. The results of this comparison are indicated in Table VIII.

TABLE VII

DETERMINATION OF KNOWN AMOUNTS OF AMINO NITROGEN ADDED TO PEPTONE WATER

SAMPLE	MG. PER 100 C.C.			
	UNBUFFERED PEPTONE PLUS GLYCINE		BUFFERED PEPTONE PLUS GLYCINE	
	VAN SLYKE	FOLIN	VAN SLYKE	FOLIN
1	9.20	8.60	9.15	9.15
2	9.75	7.85	9.30	9.15
3	8.95	8.70	9.30	8.75
Average	9.30	8.65	9.24	9.02
Theoretical	9.25		9.23	

TABLE VIII

NH_2–NH_3 NITROGEN IN VEAL INFUSION BROTH. MG. PER 100 C.C.

SAMPLE	BROTH NO. 1		BROTH NO. 2	
	VAN SLYKE SHAKEN 5 MIN.	FOLIN	VAN SLYKE SHAKEN 10 MIN.	FOLIN
1	34.60	41.30	60.20	59.7
2	36.00	40.25	60.30	58.8
3	36.20	40.50		
4	37.30			
5	44.15*			

*Shaken ten minutes.

The results in Table VIII fail to show as satisfactory agreement between the methods as was obtained with the peptone water media. The broth was not freed from ammonia, but since this reacts with the reagents of both methods, it does not vitiate the results so far as a comparison is concerned. As is indicated by Sample No. 5 of Broth No. 1 and the results with Broth No. 2, a ten-minute shaking period in the Van Slyke method yields a larger amount of amino nitrogen. Furthermore, this amount agrees better with the results obtained by Folin's method. If one wishes the actual amount of amino nitrogen present it is necessary to determine a sufficient reaction time for the particular medium. For a simple comparison of proteolysis at various time intervals, an arbitrary standard shaking period may be sufficient.

The two methods were now applied to the determination of amino nitrogen in veal infusion broth cultures of hemolytic streptococci. One-half cubic centimeter of an eighteen-hour veal infusion broth culture was added to each of two flasks containing 50 c.c. sterile veal infusion broth. After twenty-four and forty-eight hours' incubation at 37° C., a flask was removed for amino nitrogen and ammonia determinations. Stained smears established purity and growth. In order to ascertain the effect upon the two methods of not removing the ammonia, determinations were made before and after the ammonia

was removed. Ammonia was determined by the aeration method of Folin.[10] The amino nitrogen determinations after removal of free ammonia were made on samples following the aeration process. They, therefore, were saturated with sodium chloride. Table IX shows the collected data.

As will be seen the results by the two methods compare well with one another. So far as comparable results go, the presence of free ammonia has little effect. It reacts equally with both methods. If one adds together the NH_2 nitrogen obtained after removal of the ammonia and the NH_3 determined by the aeration process, the result compares favorably with the NH_2-NH_3 nitrogen obtained by either method before removal of the ammonia. The theoretical error introduced by utilizing the sample from the aeration process for the determination of NH_2 nitrogen is negligible.

TABLE IX

NH_2-NH_3 NITROGEN CONTENT OF BROTH CULTURES BEFORE AND AFTER REMOVAL OF AMMONIA. MG. PER 100 C.C.

MATERIAL	BEFORE REMOVAL OF NH_3					
	24 HR. INCUBATION			48 HR. INCUBATION		
	VAN SLYKE NH_2-NH_3	FOLIN NH_2-NH_3	NH_3 AERATION	VAN SLYKE NH_2-NH_3	FOLIN NH_2-NH_3	NH_3 AERATION
Control unincubated	57.0	50.0	8.9	57.2	46.8	8.5
Control incub. 37° C.	56.0	55.7	8.9	53.0	48.4	8.5
Culture Hemo. No. 2	67.5	66.6	15.0	65.0	69.0	14.9
Culture No. 827	67.2	67.0	15.5	63.5	65.8	15.0

MATERIAL	AFTER REMOVAL OF NH_3			
	24 HR. INCUBATION		48 HR. INCUBATION	
	VAN SLYKE NH_2	FOLIN NH_2	VAN SLYKE NH_2	FOLIN NH_2
Control unincubated	—	—	45.0	42.2
Control incub. 37° C.	45.7	—	—	47.7
Culture Hemo. No. 2	52.7	—	55.7	51.5
Culture No. 827	47.0	—	53.2	53.3

COMPARISON OF THE VAN SLYKE, FOLIN, AND SÖRENSEN METHODS FOR AMINO NITROGEN DETERMINATION IN BACTERIOLOGIC MEDIA

Since the Sörensen method[1] for the determination of amino nitrogen has been extensively used by bacteriologists in previous years, we believed it worth while to compare it with the two newer methods. In order that the formol titration should compare as favorably as possible with the other two methods, we adopted for this titration the newer technic of Brown's[11] Method "B."

The unselected successive results with the three methods on veal infusion broth and on 1 per cent peptone water are shown in Tables X and XI. Ammonia was removed from the veal infusion by treatment with permutit. The peptone water was buffered to P_H 7.8 with phosphates. Nessler's reagent failed to show appreciable ammonia in this medium.

Tables X and XI show a wide variation in the results of the three methods. The Folin method shows 16.8 per cent less amino nitrogen than the Van Slyke, while the Sörensen results are 9.3 per cent below those of the Folin method. However, the maximum deviation from the mean of the individual

samples in the Van Slyke (2.1 per cent) and Folin (2.8 per cent) methods is acceptable. The Sörensen results show a greater deviation (9.9 per cent) from the average.

Comparison between the methods on the buffered peptone water is even less satisfactory. Here there is an average difference of 24.5 per cent between the Van Slyke and Folin methods. The Sörensen results are 14.9 per cent lower than those of the Folin method. The maximum variation of the individual samples from the average is greater (7.9 per cent) here for the Van Slyke, but somewhat less (2.3 per cent) for the Folin method. The deviation from the average in Sörensen's method is greater (18.2 per cent) than appeared with the veal infusion. The presence of phosphates is known to affect the formol titration, but Brown's technic supposedly overcomes this difficulty.

TABLE X

AMINO NITROGEN IN STERILE VEAL INFUSION BROTH

SAMPLE	VAN SLYKE MG. PER 100 C.C.	FOLIN MG. PER 100 C.C.	SÖRENSEN MG. PER 100 C.C.
1	49.6	42.20	42.0
2	50.5	42.10	38.5
3	51.5	41.85	38.5
4	50.5	42.55	35.0
5	51.5	40.95	37.0
6	50.4	42.90	
Mean	50.7	42.15	38.2

TABLE XI

AMINO NITROGEN IN STERILE BUFFERED PEPTONE WATER

SAMPLE	VAN SLYKE MG. PER 100 C.C.	FOLIN MG. PER 100 C.C.	SÖRENSEN MG. PER 100 C.C.
1	34.55	24.63	17.5
2	33.60	25.60	21.0
3	30.80	25.13	18.9
4	33.15	24.84	23.8
5	36.00	25.75	24.5
6	32.00	25.00	21.0
7	----	----	23.1
Mean	33.35	25.15	21.4

We are unable to explain the variation between the methods on the same medium. The Folin method is, of course, based upon a comparison between a known standard amino acid solution and the unknown. The standard we used was the glycine solution recommended by Folin, and the sole guarantee of its accuracy rests upon the weighing of the glycine. This was done with great care on an analytical balance. In order to check this standard amino acid solution to some degree, we determined the amino nitrogen content of the standard glycine solution by both the Van Slyke and Sörensen methods.

The theoretical content of the glycine solution was 100 mg. of amino nitrogen per 100 c.c. of the solution. The average result by Van Slyke's method was 105 mg., while the Sörensen titration showed 98.2 mg. If the standard glycine solution used actually contained 105 mg. of amino nitrogen, then the Folin results should be increased. The Folin and Van Slyke results would then be more comparable, but the divergence between the Folin and

Sörensen results would be greater. On the other hand, the Folin method considers the careful weighing of the glycine as accurate, and glycine is not well determined by the Van Slyke method. We prefer, therefore, to consider the results as they stand.

DISCUSSION

From the above tabulated data it is not possible to decide which of the three methods determines the content of amino nitrogen most accurately. As a general rule, however, the bacteriologist is more interested in variations in the amino nitrogen over a certain period of time than he is in the absolute amount present. For this purpose probably any one of the three methods could be used.

The Van Slyke method has been popular because of its speed. We believe that where only a few determinations are to be run, and the results must be known as soon as possible, that this method is the one of choice. Certain precautions and attentions to detail are necessary in order that acceptable consecutive results may be obtained. A definite, uniform amount of purified caprylic alcohol must be used throughout the determinations. So also a definite shaking speed and shaking period must be adhered to throughout. The same sample will not show the same amount of amino nitrogen if shaken for three minutes and again ten minutes, nor will it show the same amount if the speed of shaking is once fast and then slow.

What has been said applies to both the blank on the reagents and the actual determinations. They must all be manipulated in exactly the same manner. It is to be remembered that the actual amount of nitrogen gas measured is very small, and that a slight error in measurement will be magnified greatly in the computations. Under these conditions the method has given acceptable successive results in our hands. We are unable to explain why DeBord[7] and Lamson[4] failed to get satisfactory checks in the main tests when they used a purified alcohol and obtained consistent checks in the blank determinations.

Where a large number of determinations are to be made and one can wait until the next day for the results, the Folin method is to be preferred. Only one determination can be made at a time with the Van Slyke apparatus, while with the Folin method a large series may be set up and the reagents pipetted for all tubes at one time. A large series, then, may be determined in less time than with the Van Slyke procedure even though they must sit overnight before reading.

This method has the advantage of employing the colorimeter which is widely available, and which can be used for other purposes than the determination of amino nitrogen. The Van Slyke apparatus is very fragile, and is apt to be broken with the press of work, causing considerable inconvenience. The apparatus cannot, of course, be used for any other purpose. Our results indicate that the Folin method gives more uniform results than the Van Slyke. There appears to be less difference between consecutive results (about 4 per cent) and less variation from the average (about 3 per cent).

The Sörensen method requires accurate titrations between very accurate

P_H limits. Some speed in the determination is necessary to prevent loss of CO_2. It is a longer, more involved method than the other two, and our results appear less satisfactory with it. The maximum variation (about 22 per cent) between consecutive results, and the deviation from the average (about 14 per cent) are greater than with either one of the other methods.

SUMMARY

1. With certain precautions, particularly the use of a definite amount of purified caprylic alcohol, the Van Slyke method for the determination of amino nitrogen values gives acceptably consistent results on peptone water and veal infusion broth.

2. A comparison of the Van Slyke, Folin and Sörensen methods for amino nitrogen determination indicates that the Folin method is the most consistent, and is best suited for a large number of determinations.

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COLORED GLASS STANDARDS*

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I. BLOOD SUGAR

THE use of colored glass standards in place of variable solutions is much desired for the small laboratory. The securing of correct standard solutions by those whose training or equipment does not permit the making of them is a very real problem, doubly so where the solutions do not keep for any length of time. In the hope of performing some such service, particularly for our own students, we have cooperated with the Klett Manufacturing Company in the preparation of glass standards.

The standard most desired is that for blood sugar, and the one which has given us the most trouble. We followed Folin's new method[1] and found that scarcely two standards prepared from the same known glucose solution gave exactly the same color. We found it necessary, if we wished to get anything like the same shade of blue, to duplicate our procedure exactly. Calibrated apparatus, of course, was used throughout.

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Received for publication, August 4, 1928.

Immediately after introducing the glucose solution and alkaline copper tartrate into the sugar tube, our procedure was to heat it in the boiling bath for exactly six minutes; to transfer it to a cold water-bath for exactly four minutes; to add the molybdate, dilute, and read. If the solution is diluted at once the color fades rapidly. In general, the longer the molybdate mixture stands before dilution, the more blue the diluted solution and the longer it takes to fade. This Dr. Katayama had observed in the routine analysis of our blood sugars. Since he found that with twenty or thirty tubes the fading in color was so pronounced as to prevent proper matching of colors, he regularly waited fifteen minutes before diluting. Our waiting periods ranged from no minutes to three days. A glass, that matched perfectly 15 mm. of solution which had been allowed to stand twenty minutes before diluting, appeared very gray and purplish against the deep bright blue of a solution that had stood three days after the addition of the molybdate. The latter solution underwent no observable fading for twenty-four hours after diluting. Standards diluted in less than twenty minutes were green and gray against the glass; those later than twenty minutes were too blue.

In the hundreds of glucose solutions prepared, not one was found that within the five-minute period immediately following dilution, ever gave consistent or certain readings. The color is exceedingly variable. At the beginning there is more red in the color; this rapidly disappears. This shift in colors is followed by a period, usually of not more than five minutes, during which the color is stable. Then the blue fades, leaving green and gray to predominate.

This variation in color is of more than theoretic interest. If a blood such as an emergency specimen is brought in for analysis when the routine specimens have just received the molybdate, is it permissible to hold them at that stage, to run the new specimen through and to match it with the others? It is very convenient; it is economical both of time and of reagents. We did just this. The same filtrate was used and the analyses made simultaneously. The one matched against the standard that had waited gave a sugar of 0.18 per cent. The other matched against a fresh standard prepared with it gave 0.25 per cent.

The rate of fading after dilution is important in determining how soon the colors must be matched. To take a single instance: the unknown reads 10.6 against both the glass standard and the solution standard set at 15 mm. for twelve minutes. Thirty minutes after diluting, the unknown reads 8.7 to 8.9 against the standard set at 15. Against the glass it reads 13. Depending on when and against what the reading is taken, the sugar in the blood would be read as 0.141, 0.170, or 0.115 per cent.

Because of the uncertainty of our results with Folin's modification, we have returned to the old Folin-Wu method.[2]

This method, while not showing the extreme variations encountered with the new Folin reagents, is subject to the same limitations.

With the glass now available for use, the unknowns must be boiled vigorously six minutes and cooled for three minutes. After the addition of molyb-

date, fifteen minutes must elapse before diluting, and then another five minutes before reading. Color comparisons must be made within the following ten minutes.

The blue color developed depends upon many unknown factors. Where the glass standard is used the limitations are more obvious than with solutions. Since it is possible to produce an almost infinite variety of blue colors with *standard glucose solutions*, the glass standard can give accurate results only when the directions are obeyed explicitly.

Wherever the glass disc is used, *each* worker should determine his own corrective factor. Working conditions, such as, light and temperature, quality and age of reagents used, and personal variations in color comparisons will all affect the factor. Nevertheless, we venture to say that there is probably much less error in using a standardized glass, together with the factor determined by the maker, than in using standard solutions bought from ordinary commercial houses or prepared by the average technician.

The chemicals employed in preparing the reagents used may vary the factor tremendously. The copper reagent should be a clear deep blue. Whenever much sediment appears or the blue color seems faded, poor results will be obtained. When our molybdate is fresh (at least for ten to fourteen days after preparation), it gives less blue with cuprous oxide than when it is a month old.

In attempting to standardize the glass we had repeated setbacks. Just as we were about to draw conclusions, all our known solutions would suddenly shift their colorimetric reading 1 to 2 mm. higher than they had been running. Finally we discovered that this difference was evident every time new reagents were prepared. The new molybdate was found to be the troublesome factor. At present we recommend that the phospho-molybdate reagent be prepared two or three weeks before use. This solution, however, will stand further investigation before any decision can be given as final. Wu has already observed that the complex phospho-molybdic-tungstic acids give different colors or different shades on slight variations in conditions.[3] Since these complex acids may be easily reduced, giving a good blue color, they have been regarded as admirable color reagents. They are, but not in the hands of a careless or ignorant technician.

It is impractical to publish pages upon pages of figures to show exactly why the molybdate is allowed fifteen minutes before dilution. We do not claim that it is even ideal, but it is the shortest period that we have been able to find that resulted in a color stable enough to permit the reading of several unknowns. The actual number of determinations that can be run at once depends upon the speed of the worker in color matching.

The temperature of the cold water-bath affects the final color. Duplicates were run, some being cooled in water kept at 20 to 24° C. and the others in water containing ice. It was found that those cooled at 20° C. gave an excellent match with the glass disc. Those cooled in ice water were such a clear green-blue that the glass seemed cloudy and distinctly purplish against them.

TABLE I

ROUTINE BLOOD SUGARS (FOLIN-WU METHOD)

BLOOD NUMBER	MINUTES AFTER DILUTION	COMPARISON WITH GLASS						COMPARISON WITH SOLUTION		
		READING (AVERAGE)	REMARKS	CALCULATED SUGAR, G/100 C.C.				READING (AVERAGE)	REMARKS	CALCULATED SUGAR G/100 C.C.
				UNCORRECTED	C.F. = 1.20	C.F. = 1.14	C.F = 1.05			
Standard	6	12.5	C.F. = 1.20							
1 a	8	17.5		0.085	0.071			20.8		0.072
1 b	10	11.0		0.136	0.114			13.1		0.114
1 c	11	9.5		0.158	0.132			10.6		0.141
2	13	12.5		0.120	0.101			14.7		0.102
3 a	15	24.6	Solution gray	0.061	0.051	0.054		28.0		0.054
Standard	16	13.2	Excellent match; C. F. = 1.14							
3 b	17	7.9	Easy to match, but solution too blue	0.190	0.159	0.167		8.9	Much harder to match than with glass	0.169
4	18	13.6		0.110	0.092	0.097		15.4		0.097
5	20	17.3		0.087	0.073	0.076		19.6		0.077
6	22	14.0	Good match	0.107	0.089	0.094		15.4		0.097
7	23	14.3	Somewhat hard to match	0.105	0.088	0.092	0.100	15.7		0.095
8	25	11.4	Solution gray	0.132	0.110	0.115	0.125	12.1		0.124
9	27	15.7	Solution quite gray	0.096	0.080	0.084	0.091	17.9		0.084
10	28	14.0	Solution quite gray	0.107	0.089	0.094	0.102	14.8		0.101
11	30	19.8	Solution very gray	0.076	0.064	0.067	0.072	21.1	Too green for good match	0.071
12	31	18.2	Solution very gray	0.083	0.067	0.073	0.078	19.0	Poor match	0.079
Standard	35	14.3	Match difficult; C.F. = 1.05							

Unless the worker prepares his own series of corrective factors, the color comparisons should be confined to a period of five to ten minutes following the five minutes of waiting for a stable color. The factor as determined by us for this period is 1.2 (multiply the colorimeter reading by this and apply the corrected figure to the usual calculation). If conditions are just right, it is possible to make decent comparisons for as much as twenty minutes after dilutions, but no one corrective factor can be applied to such a series of readings. The glass is used without either cup or plunger.

Table I shows the sort of results that we have invariably obtained when reading unknowns against both glass and standard solution. That we could get such excellent checks was a great surprise to us since we believed before we started that our figures would demonstrate that the glass disc could not be used in routine work.

Table II shows the typical shift in color seen in the standard glucose solution.

TABLE II

CORRECTIVE FACTOR FOR SUGAR DISC

TIME AFTER DILUTING	READING OF STANDARD SOLUTION*	REMARKS	CORRECTIVE FACTOR
5 minutes	12.3-12.7	Fair match	
6 minutes	12.4		1.21
7 minutes	12.5	Excellent match	1.20
10 minutes	12.7		1.18
12 minutes	12.9		1.16
14 minutes	13.0	Harder to match	1.15
15 minutes	13.1		1.14
20 minutes	13.4	Gray	1.12
25 minutes	——	Too gray to match	

*The worker must allow for the fact that bubbles of CO_2 may collect on the plunger, otherwise that will cause a "shade" to appear. Between readings the plunger should be raised above the level of the fluid in the cup.

Our standard glucose solution is prepared with saturated benzoic acid. This acid affects the depth of color produced. Three standards were prepared: (1) One hundredth per cent glucose in distilled water; (2) one hundredth per cent glucose in half saturated benzoic acid; and (3) the same in fully saturated benzoic acid. With (1) set at 15, (2) read 13.4, and (3) read 12.4. This experiment was repeated several times and gave always the same sort of difference.

Exposure to light before or after diluting causes a fading of the color. For our tests we used a 500-Watt daylight Mazda bulb. The solutions were kept about a foot from the bulb. The temperature in no case increased more than 0.3° C. For example ten minutes exposure before diluting gave a reading of 16.4 against the control at 15. Exposure for five minutes after diluting gave a reading of 16.4, 16.8 against the control at 15. The exposed solution was definitely gray.

The addition of xylol or toluol in minute amounts for two days at refrigeration temperatures had no appreciable effect upon the final blue color.

Readings on high or low sugars against the glass disc cannot be regarded as accurate any more than against a standard solution of like intensity. The means to solve this difficulty is the usual colorimeter correction curve which must be prepared by the individual workers.

II. NITROGEN STANDARD*

The ammonium sulphate standard, containing 1 mg. N. in 100 ml. as used, is employed for Urea N. and total N. in urine, for Urea N. and nonprotein N. in blood, for proteins in milk, and for any Micro-Kjeldahl method where Nesslerization is the final step.

The chemicals used in the preparation of the solution for standardizing the glass follow:

Ammonium sulphate – Baker's Analyzed
Nessler's Solution – (Bock-Benedict formula)
Mercuric iodide – Merck's, Blue Label
Potassium iodide – Merck's, Blue Label
Sodium hydroxide – Merck's, Blue Label

The yellow color obtained is remarkably stable. It darkens noticeably the first half hour after preparation. After this the color intensity remains practically the same for hours. On prolonged standing the ammonium sulphate standard acquires an orange tint which makes matching with unknowns somewhat difficult. The unknowns we have tested show no appreciable increase in color on standing after Nesslerization.

The standard solution darkens slowly so that after eighteen hours its reading against the glass standard will have dropped 0.5 to 1.0 mm., and the orange tint may be very pronounced. The addition of 1 drop of 10 per cent HCl and 2 drops of amyl alcohol, either alone or together, had no apparent affect upon the orange color.

Since exposure of the nitrogen standard in an open beaker to a 500 watt daylight bulb deepens the color appreciably, we recommend that the solution be kept away from strong light whenever the worker checks the glass standard against his standard solution. The glass is used without plunger or cup.

Since the standard solution does not read 15 against the glass, a corrective factor is necessary. If possible *each* worker should determine his own. Where this is out of the question, we suggest 0.95 or 0.96 as the corrective factor. The reading of the unknown is multiplied by this factor and the corrected reading applied to the regular calculation. See Tables III and IV.

TABLE III

NITROGEN STANDARD

MINUTES AFTER PREPARATION	READING	REMARKS	CORRECTIVE FACTOR
2	16.0-16.1	Glass a little too yellow	0.94-0.93
4	16.0		0.94
9	15.7		0.95
14	15.7		0.95
40	15.6	Faint orange in standard	0.96
55	15.3-15.5		0.98-0.97
140	15.4-15.5		0.97
155	15.6	Distinctly orange	0.96
18 hours	14.0	Very orange against yellow glass	1.07

*The methods used for nonprotein nitrogen and urea in blood are those described by Victor C. Myers in "Practical Chemical Analysis of Blood," 1924, p. 43-46.

TABLE IV

UREA ROUTINE

BLOOD NO.	TIME AFTER NESSLERIZATION	COMPARISON AGAINST GLASS		COMPARISON AGAINST SOLUTION	
		R	CALCULATED VALUE C.F. = 0.96	R	CALCULATED VALUE MG./100 C.C.
Standard	35 minutes	15.5	C.F. = 0.96		
1	10 minutes	16.1	14.7	15.0	15.0
2	17 minutes	22.6	10.4	21.4	10.5
3	20 minutes	23.0	10.2	22.2	10.1
4	22 minutes	18.8	12.5	17.7	12.7
5	25 minutes	15.2	15.6	14.6	15.4
6	27 minutes	17.8	13.2	17.0	13.2
11	30 minutes	16.7	14.0	16.2	13.8
12	32 minutes	28.6	8.2	27.0	8.3
11	again after 2 hours	16.5	14.2		
1	again after 1 hour	16.0	14.7		

III. URIC ACID*

For our purpose we employ the method of Brown.[4] Two standards, 0.05 and 0.025 are necessary. The blue color here varies rapidly.

With a method as simple as this, we see no reason for attempting to run a long series at the same time. Two or three prepared at five or ten minute intervals can be matched against the glass standards with as great accuracy as is possible with any standard solution. We strongly recommend that the period of color development be increased to twenty-five minutes instead of twenty unless turbidity intervenes. At the end of the twenty-minute period we have invariably found the standard solutions still increasing rapidly in color. The best time for matching is about thirty minutes after starting color development. From about twenty-six to thirty-three minutes after the start, the standard solutions oscillate about 15 when compared to their proper glass standards. The plunger is best used with the glass, otherwise there will be too much "shade" in the unknown solution.

If color comparison is made at the end of the twenty minutes, a corrective factor is necessary. (Consult Tables V, VI, VII.) There is a progressive deepening of color until the solution becomes too gray to match.

Exposure to light causes a fading of the blue color.

TABLE V

URIC ACID: 0.05 STANDARD

TIME AFTER SETTING UP	READING	REMARKS	CORRECTIVE FACTOR
20 minutes	16.8-17.0	Good match	0.89-0.88
22 minutes	15.8		0.95
24 minutes	15.1		0.99
26 minutes	15.1-15.4		0.99-0.97
28 minutes	15.2		0.99
30 minutes	14.8-15.0		1.01-1.00
32 minutes	15.0		1.00
34 minutes	14.3	Harder to match	1.05

*The author is indebted to Dr. Ichiro Katayama, Claire Murphy, and Lena Halpern for preparation of the standard solution.

TABLE VI

URIC ACID: 0.05 STANDARD

TIME AFTER SETTING UP	READING	REMARKS	CORRECTIVE FACTOR
20 minutes	17.0	Good match	0.88
20½ minutes	16.7		0.90
21 minutes	16.3		0.92
21½ minutes	15.9		0.94
22 minutes	15.8		0.95
23 minutes	15.7		0.95
24 minutes	15.2		0.99
25 minutes	15.1		0.99
26 minutes	15.0		1.00
28 minutes	14.8-15.0		1.01-1.00
29 minutes	14.8-15.0		1.01-1.00
30 minutes	14.8		1.01
32 minutes	14.8	Harder to match	1.01

TABLE VII

URIC ACID: 0.025 STANDARD

TIME AFTER SETTING UP	READING	REMARKS	CORRECTIVE FACTOR
19 minutes	16.8-17.0	Poor match	0.89-0.88
22 minutes	15.9		0.94
24 minutes	15.8		0.95
26 minutes	15.4	Good match	0.97
28 minutes	15.3		0.98
30 minutes	15.2-15.4		0.99-0.97
31 minutes	15.2		0.99
33 minutes	15.2		0.99
34 minutes	15.1		0.99
36 minutes	14.8	Harder to match	1.01
38 minutes	14.4		1.04
41 minutes	14.0		1.07
44 minutes	13.9	Very hard to match	1.08

SUMMARY

Glass standards for blood sugar, urine and blood nitrogen, and blood uric acid have been described and their limitations noted.

The glass standards described offer a solution of the standard problem, albeit with reservations, and what is more important, they offer to the laboratory investigator the means of studying the color changes and their variations in the methods upon which he depends.

The author is indebted to Mr. Klett and to his assistant, Mr. Daniel, for their untiring efforts in preparing dozens of trial glasses for these experiments.

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STUDIES ON ANTIGEN FOR THE KAHN TEST*

II. A Sensitizing Reagent

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ANTIGENS used in serum tests for syphilis may be said to have two outstanding limitations. In the first place, they lack uniformity in sensitiveness, even when a standard method of preparation is used; this is due to the fact that the heart muscle from which the antigen is prepared varies markedly in its content of antigenic lipids. In the second place, antigens appear in general to be insufficiently sensitive, no antigen thus far prepared being capable of detecting all cases of syphilis. The development of antigens more adequately adjusted to diagnostic ends has been somewhat delayed by a meager knowledge of the factors governing antigen sensitiveness. During the past several years the studies on antigen for the Kahn test, carried out in this laboratory, have been directed toward overcoming both of these limitations. A preliminary report dealing with the uniformity of standard antigen has recently appeared.[1] The present study deals with two series of experiments on antigen sensitiveness. In the first series, attempts were made to determine the relative amount of antigenic material present in the discarded by-products of antigen preparation. The results obtained in these first studies were then applied in a second series of experiments in the course of which a sensitizing reagent was developed capable of increasing antigen sensitiveness without apparently affecting specificity.

It will be recalled[2] that the preparation of antigen for the Kahn test involves two basic extractions of powdered heart muscle, a series of extractions with ether followed by a single extraction with alcohol, and that it is this alcohol extract which, after cholesterolization and standardization, is employed as the antigen. It was early observed that the extent of the ether extractions of heart muscle markedly affects the sensitiveness of the final product. Excessive extraction with ether tends to reduce antigen sensitiveness, while deficient extraction with ether, within certain limits, increases antigen sensitiveness. Thus in the preparation of standard antigen, 25 gm. of heart muscle are extracted successively with 100, 75, 75, and 75 c.c. of ether, while in the case of the "special" more sensitive antigen that was prepared for use in the presumptive procedure, the successively employed ether amounts for the same quantity of heart muscle were 50, 50, 40, and 40 c.c. Since much of the lipid material that would be removed by a preliminary ether treatment will go into solution in the alcohol if the ether treatment is omitted or reduced, the antigen prepared according to the second, "special" method contains more ether extractives than the first, and is therefore

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Received for publication, July 17, 1928.

more sensitive. It was further reported from earlier experiments than when 25 gm. of heart muscle are extracted with larger volumes of ether than those employed in the preparation of standard antigen, the resulting reduction in the sensitiveness of the antigen can be overcome by adding to the antigen ether extractives in concentrated form. Such extractives can also be utilized in correcting antigens which are similarly less sensitive than standard antigen because of inherent poverty in antigenic lipids of the heart muscle from which they are prepared.

The fact that the ether extractives of heart muscle contain antigenic lipids prompted a study of these extractives with a view to their utilization in the Kahn test. At first it seemed desirable to prepare an acetone insoluble extract of these ether extractives and to test its degree of sensitiveness and specificity with syphilitic and nonsyphilitic sera. The antigenic value of a secondary alcoholic extract of heart muscle seemed also worth investigating. Finally, it seemed desirable to prepare an alcoholic extract of ether extractives. The studies carried out with this last extract ultimately led to the preparation of an antigen sensitizing reagent which under proper conditions is capable of increasing antigen sensitiveness.

I. ANTIGENIC VALUE OF THREE SPECIAL EXTRACTS

A. *An Acetone Insoluble Fraction of the Ether Extractives of Heart Muscle.*—The ether extractives obtained in the preparation of antigen from 50 gm. powdered beef heart were evaporated nearly to dryness. The small amount of water which collected at the bottom of the evaporating dish was removed by means of a capillary pipette, and the lipid residue was then dissolved in 15 c.c. of ether and the solution filtered. When 10 c.c. of the filtrate were measured into a 250 c.c. cylinder and 200 c.c. acetone added, a fine precipitate appeared instantly. After vigorous shaking the particles became larger and soon settled to the bottom of the cylinder leaving the acetone solution practically clear. The supernatant acetone was decanted, and the lipid residue taken up in 50 c.c. of 95 per cent alcohol. Extraction was permitted to take place for thirty minutes at room temperature with frequent shaking, and the alcoholic mixture was then filtered. To the filtrate were added 6 mg. cholesterol per cubic centimeter, the cholesterol being dissolved by warming the mixture in a water-bath. After filtration, the extract was titrated, employing the same method as is used for the titration of standard antigen. It was then tested with syphilitic and nonsyphilitic sera.

Table I illustrates the comparative sensitiveness of the acetone insoluble fraction of the ether extractives and of standard antigen. It is evident from this table that the fraction not only has antigenic properties but is actually comparable in sensitiveness and specificity with standard antigen. It was noticed, however, that the negative reactions appeared more cloudy than in the case of standard antigen. An attempt was made to overcome this shortcoming by a readjustment of the amount of salt solution used in preparing the antigen suspension. When, however, the titer was increased to 1 + 2.1, the negative reactions still appeared somewhat cloudy, and furthermore it

TABLE I

COMPARATIVE SENSITIVENESS OF ACETONE INSOLUBLE FRACTION OF ETHER EXTRACTIVES, AND OF STANDARD ANTIGEN

SERUM NO.	ACETONE INSOLUBLE FRACTION TITER 1 + 1.9			STANDARD ANTIGEN TITER 1 + 1.1		
	1	2	3	1	2	3
1	–	–	–	–	–	–
2	–	–	–	–	±	++
3	–	–	±	–	–	–
4	–	±	±	–	–	–
5	++	++	+++	+++	++++	++++
6	++	++++	++++	++++	++++	++++
7 – 10	++++	++++	++++	++++	++++	++++

was observed that the antigen gave atypical reactions with four-plus sera, or reactions in which the third tube of the regular test showed very weak precipitates. The results are illustrated in Table II.

When the acetone insoluble fraction was diluted with cholesterolized alcohol (10 to 25 per cent dilution), there was observed a slight increase in sensitiveness. When the fractions were added, either undiluted or after dilution with cholesterolized alcohol, to standard antigen in a 50/50 proportion, the sensitiveness of the final mixtures was not markedly different from that of standard antigen, except that the antigen containing the acetone insoluble fractions gave atypical reactions.

TABLE II

ATYPICAL REACTIONS GIVEN BY ACETONE INSOLUBLE FRACTION

SERUM NO.	ACETONE INSOLUBLE FRACTION TITER 1 + 2.1			STANDARD ANTIGEN TITER 1 + 1.1		
	1	2	3	1	2	3
1	–	–	–	–	–	–
2	++++	++++	±	++++	++++	++++
3	++++	++++	+	++++	++++	++++
4	++++	++++	+++	++++	++++	++++
5	++++	++++	++	++++	++++	++++
6	–	–	–	–	–	–

The results obtained with the acetone insoluble fractions of the ether extractions did not seem to warrant further detailed studies of this fraction, because, although it possesses a degree of sensitiveness approximating that of standard antigen, it is somewhat troublesome to prepare, it does not appear to give a sharp titer, and it has a tendency to give atypical reactions.

B. *A Secondary Alcoholic Extract of Heart Muscle.*—Our next problem was to determine whether a secondary alcoholic extract of powdered heart muscle that had already been treated to the first alcoholic extraction in the standard preparation of antigen, would show antigenic properties. This had been attempted before with negative results,[3] but it seemed worth while to repeat the experiments. Powdered beef heart was used from which the original (primary) alcoholic extract had been filtered without the aid of suction, through qualitative filter paper in a glass funnel. This method of filtration permitted a considerable amount of the alcoholic filtrate to adhere

to the residue. For the secondary extract, 50 gm. of this residue were extracted with 100 c.c. of 95 per cent alcohol for one hour, in a water-bath at 60° C., and finally for twenty-four hours at room temperature. When this secondary extract was allowed to stand after filtration, a precipitate separated slowly from the liquid and was removed by repeated filtrations. After five days standing, the extract showed only a trace of a precipitate and was then cholesterolized, 6 mg. of cholesterol being dissolved in each cubic centimeter of extract.

The secondary alcoholic extract was titrated in the usual manner and tested for sensitiveness with syphilitic sera of different potency, standard antigen being used as a control. The results with several sera are given in Table III. It is evident from this table that the secondary alcoholic extract was considerably less sensitive than standard antigen.

TABLE III

COMPARATIVE SENSITIVENESS OF SECONDARY ALCOHOLIC EXTRACT OF HEART MUSCLE, AND OF STANDARD ANTIGEN

SERUM NO.	SECONDARY ALCOHOLIC EXTRACT TITER 1 + 1.2			STANDARD ANTIGEN TITER 1 + 1.1		
	1	2	3	1	2	3
1	++++	++++	++++	++++	++++	++++
2	−	±	±	+++	++++	++++
3	−	±	−	+	++	+++
4	±	+	+	−	+	++
5 – 6	−	−	−	−	−	−

The question arose as to whether the antigenic powers of the secondary alcoholic extract were not in part due to the occlusion of some of the original extract antigen in the beef heart residue. At least 30 per cent of the alcoholic filtrate remains adhering to the particles when the filtration process is carried out in an ordinary glass funnel. In order to throw light on this question, standard antigen was prepared from 100 gm. of beef heart, and was filtered as completely as possible by means of suction through a Buchner funnel. To the powder residue were then added 250 c.c. of alcohol. Extraction was carried out as previously described; namely, for one hour at 60° C. followed by twenty-four hours at room temperature. After filtration and cholesterolization, this secondary extract was practically free from antigenic properties. As will be shown later, when "sensitizing reagent" was added to this secondary alcoholic extract, the resulting product showed some antigenic properties with syphilitic serum. The precipitates were, however, extremely fine and difficult to detect.

To summarize our findings with secondary alcoholic extracts of heart muscle, it appears that such extracts have very little antigenic material, especially when the heart muscle has previously been freed from the original antigen by filtration with suction.

C. *An Alcoholic Extract of Ether Extractives of Heart Muscle.*—Our studies were next directed to the preparation of an alcoholic extract of the concentrated ether extractives of heart muscle. About 500 c.c. ether extract

collected in the course of antigen preparation from 50 gm. of powdered beef heart, were evaporated nearly to dryness with the aid of an electric fan. When practically all of the ether had evaporated, a few globules of water, which separated from the lipid mass and settled to the bottom of the dish, were removed with a capillary pipette. The lipid residue was taken up in 50 c.c. of absolute alcohol, and the mixture was heated for ten minutes in a water-bath at 56° C. with frequent shaking. The mixture was then placed in the ice box (7° C.) for one hour, and was filtered while cold. The clear yellow solution became cloudy after standing about one-half hour at room temperature. It was refiltered and cholesterolized (6 mg. of cholesterol per c.c.).

It was observed that this extract was of little practical value when used directly as an antigen. Indeed, it was impossible to establish its "titer." Even when as much as 3 to 5 c.c. of salt solution were added to 1 c.c. of the extract, there resulted a lipid suspension the particles of which were not entirely dispersable in additional salt solution or in negative serum. When, however, the extract was added to standard antigen in a proportion of about 2 or 3 parts of extract to 100 parts of antigen, it markedly increased the sensitiveness of the latter. Table IV gives results of comparative tests with standard antigen alone and standard antigen plus 3 per cent of the special extract.

TABLE IV

EFFECT ON SENSITIVENESS OF STANDARD ANTIGEN OF ADDING ETHER SOLUBLE, ALCOHOL SOLUBLE EXTRACT

SERUM NO.	STANDARD ANTIGEN PLUS 3% ETHER SOLUBLE, ALCOHOL SOLUBLE EXTRACT TITER 1 + 2.0			STANDARD ANTIGEN TITER 1 + 1.1		
	1	2	3	1	2	3
1 – 2	–	–	–	–	–	–
3	–	++	++++	–	–	±
4	+	++++	++++	–	–	++
5	++	++++	++++	–	+	++
6	++++	++++	++++	–	++	++++
7	+++	++++	++++	–	++	+++
8	++++	++++	++++	±	++++	++++
9 – 10	++++	++++	++++	+	++++	++++

This observation suggested that the preparation of an alcoholic solution of the ether extractives in a stable form would yield a valuable reagent for the Kahn test. A reagent of this type ("sensitizing reagent") could be used for correcting newly prepared antigens that are less sensitive than standard; it could also be used in the preparation of antigens more sensitive than standard.

II. EXPERIMENTS ON PREPARATION OF SENSITIZING REAGENT

In attempting to standardize the method of preparing the sensitizing reagent many procedures were tried. Ten minute alcohol extractions of the lipid residue from the ether extract were carried out in some cases at 56° C., and in other cases at 60° C. These extractions were followed by further

extraction in the ice box for from one to three hours. Although the resulting reagents showed good promise with respect to their antigen sensitizing properties, this method of preparation was ultimately abandoned because the extracts tended to yield precipitates on standing. These precipitates usually appeared in the form of a gummy sediment adhering to the walls of the container. On filtration, a new sediment would appear after several days' standing. It was not possible to determine whether the sediment had antigenic properties, since so small a quantity was deposited. The extract showed, however, but little variation in its sensitizing properties after repeated filtration from the sediment. Nevertheless, it was believed undesirable to accept as standard a method of preparation yielding a sensitizing reagent from which a sediment was constantly being deposited. Attempts were therefore made so to modify the method as to prepare an effective sensitizing reagent that would remain free from a precipitate.

Various modifications of the extraction procedure were tested. The lipid residue from the ether extract was extracted with alcohol for ten minutes at room temperature (instead of at 56° C. or 60° C.) followed by several hours in the ice box (7° C.). In other cases tried, the extraction for ten minutes at room temperature was followed by continued extraction in the ice box for three days. In still other cases the residue was extracted with alcohol at room temperature for three days. All these methods yielded sensitizing reagents from which sediments were deposited when the reagents were allowed to stand for several days. It was finally observed that the formation of a sediment was either markedly reduced or entirely eliminated if after the extraction of the lipid residue at about 7° C., the mixture was filtered and the filtrate placed in the incubator at 37° C. overnight. This observation led to the adoption of the following extraction method. The residue is extracted at room temperature for ten minutes and then in the ice box at about 7° C. for three or four hours. The mixture is filtered while cold, and the filtrate placed in the incubator at 37° C. for twenty-four hours.

Many varieties of heart muscle were found to be satisfactory for use in the preparation of sensitizing reagent. Several lots of "Difco" powdered heart muscle were employed with good results. Home ground sheep heart, pig heart, and beef heart were also found to produce good sensitizing reagents. The concentrated ether extract of pig heart contained a colorless fluid which collected in a layer above the usual brown residue, and which could apparently be removed without interfering with the sensitizing properties of the final alcoholic extract.

The evaporation of the ether extractives was in most cases carried out with the aid of an electric fan. In several instances, however, an electrically heated water-bath at 100° C. was employed for this purpose, while in isolated cases fan and electric water-bath were combined and the evaporation of the ether took place very rapidly. All these methods of evaporation appeared to give good results. In two instances the highly viscous lipid residue obtained after evaporating the ether was kept overnight, thus permitting oxi-

dation and partial solidification of the lipids. This treatment did not, however, interfere with the production of a good sensitizing reagent.

The treatment of the lipid residue after evaporation of the ether was also subjected to special study. In some cases, the globules of water which collected at the bottom of the evaporating dish were removed with a capillary pipette. In other cases, the water was permitted to evaporate on a water-bath at 100° C. In some instances the mixture of lipid residue and water was poured into a centrifuge tube, and after about five minutes' centrifugalization, the water which had collected at the bottom of the tube was readily removed with a capillary pipette. All these methods were found to give comparable results.

With regard to the alcohol used for the extraction process, absolute alcohol was found to be superior to 95 per cent alcohol, the former producing more sensitive sensitizing reagents. A proportion of 10 c.c. of absolute alcohol per gram of residue (or 8 c.c. absolute alcohol per cubic centimeter of residue) was found to be satisfactory. Extraction proportions of 20 or 15 c.c. of alcohol per gram of residue produced reagents which were less sensitive.

III. METHOD OF PREPARING SENSITIZING REAGENTS

On the basis of these experiments the following method was adopted for the preparation of sensitizing reagent.

1. The ether filtrate obtained as a by-product in the preparation of antigen from 25 gm. of heart muscle (about 250 c.c. of filtrate, allowing for some loss by evaporation) is refiltered to remove traces of powdered muscle, and the solvent is then evaporated with the aid of an electric fan.

2. When the volume has been reduced to about 10 c.c., the concentrated ether extract is transferred to a small, weighed evaporating dish (capacity about 20 c.c.), the transference being made complete by washing out the residue into the small dish with a little ether.

3. Evaporation is continued with the aid of the fan until there remains but a slight trace of ether odor.

4. At this stage there may separate from the dark brown lipid mass a few cubic centimeters of water. This water, which will be at the bottom of the evaporating dish, is removed by means of a capillary pipette. The lipid residue is brownish, semitransparent, and viscous.*

5. As soon as the residue is completely free from ether odor, the evaporating dish is reweighed, and the weight of the residue determined.

6. The residue is transferred to an Erlenmeyer flask (about 100 c.c. capacity). This is best accomplished with the aid of a small spatula.

7. A volume of absolute alcohol equivalent to 10 c.c. per gram of residue is added to the flask, a small amount of this alcohol being employed for rinsing the evaporating dish.

*An alternate method is to remove the water at this stage by evaporation for ten minutes on a water-bath at 100°C. (stirring with a glass rod). After evaporation, steps 5 and 6, etc., follow as outlined, but in step 7 the volume of absolute alcohol used should then be 13 c.c. instead of 10 c.c. per gm. of heated residue.

8. Extraction is allowed to take place for ten minutes at room temperature with frequent shaking of the flask. Only a fraction of the residue dissolves in alcohol, lipid masses being distributed throughout the mixture.

9. The mixture is placed in the ice box (4° to 9° C.) for three hours, during which period the bulk of undissolved matter is increased by the formation of a white precipitate.

10. The mixture is filtered while cold and the flask containing the clear dark yellow filtrate is placed in the incubator at 37° C. for twenty-four hours.

11. The clear filtrate is permitted to stand for three days at room temperature. If a precipitate forms during this test period the solution is refiltered and again incubated for twenty-four hours at 37° C.

12. The filtrate is cholesterolized with 6 mg. cholesterol per c.c. according to the usual technic.

13. The cholesterol extract, known as "sensitizing reagent" is filtered and is then ready for use.[4]

IV PROPERTIES OF SENSITIZING REAGENT

Sensitizing reagent has the property of increasing the sensitiveness of antigens that are less sensitive than standard; it can also increase the sensitiveness of standard antigen. When the amount of reagent added to antigen is less than 1 per cent, the increase in sensitiveness is usually small. As the proportion of reagent to standard antigen is increased to 2, 3, 4, and 5 per cent, the products show a graded increase in sensitiveness. Increasing the amount of reagent beyond 5 per cent usually results in a drop in the sensitiveness of the final product. This is illustrated by the following experiment:

To five 10 c.c. quantities of standard antigen were added 1.5, 3, 6, 9, and 12 per cent sensitizing reagent, respectively. The modified antigens were titrated in the usual manner, and were tested comparatively employing the 3-tube Kahn test. It was found that the addition of 1.5, 3, and 6 per cent sensitizing reagent to standard antigen resulted in a progressive increase in sensitiveness while the addition of 9 and 12 per cent sensitizing reagent resulted in a progressive decrease in antigen sensitiveness. The results of the experiment are shown in Table V.

TABLE V

EFFECT ON THE SENSITIVENESS OF STANDARD ANTIGEN OF ADDING INCREASING AMOUNTS OF SENSITIZING REAGENT*

NO. SERUM	PARTS OF SENSITIZING REAGENT ADDED TO 100 PARTS OF STANDARD ANTIGEN					
	0 TITER 1 + 1.1	1.5 TITER 1 + 1.5	3 TITER 1 + 2.1	6 TITER 1 + 2.3	9 TITER 1 + 2.5	12 TITER 1 + 2.7
1	++++*	++++	++++	++++	++++	+++
2	+++	++++	++++	++++	++++	++++
3	++	+++	++++	++++	+++	++
4	++	++	+++	++++	+++	++
5	±	++	+++	+++	++	–
6	±	++	++	+++	++	–
7	±	++	++	+++	++	–
8	–	++	++	++	–	–
9	–	–	–	–	–	–
10	–	–	–	–	–	–

*Average of 3-Tube Kahn Test.

It is interesting to note that the increase in sensitiveness effected by adding sensitizing reagent to an antigen is often enhanced by diluting the product with cholesterolized alcohol. Table VI illustrates this point, a striking increase in sensitiveness being obtained after adding 1 per cent sensitizing reagent and diluting 10 per cent with cholesterolized alcohol, but only a moderate increase in sensitiveness when 1 per cent sensitizing reagent was added alone.

TABLE VI

COMPARATIVE EFFECT ON ANTIGEN SENSITIVENESS OF ADDING SENSITIZING REAGENT ALONE AND OF ADDING SENSITIZING REAGENT PLUS CHOLESTEROLIZED ALCOHOL

SERUM NO.	UNMODIFIED ANTIGEN (UNDERSENSITIVE) TITER 1 + 1			ANTIGEN MODIFIED BY ADDITION OF 1 PER CENT SENSITIZING REAGENT TITER 1 + 1.1			ANTIGEN MODIFIED BY ADDITION OF 1 PER CENT SENSITIZING REAGENT PLUS 10 PER CENT DILUTION WITH CHOLESTEROLIZED ALCOHOL TITER 1 + 1.1		
	1	2	3	1	2	3	1	2	3
1	−	−	−	−	−	−	−	−	−
2	−	−	−	−	−	−	−	±	++
3	−	−	±	−	−	±	−	−	++
4	−	−	±	−	±	+	+	++	+++
5	−	±	+	−	±	+	±	+++	+++
6	−	±	+	−	±	++	±	+++	+++
7	−	±	+	−	++	++	−	++	+++
8	−	±	+++	±	+++	+++	+++	++++	++++
9	++	+++	++++	+	+++	++++	+++	++++	++++
10	+++	++++	++++	+++	++++	++++	++++	++++	++++

It was stated above that the secondary alcoholic extract has but slight antigenic properties. If, however, sensitizing reagent is added to the secondary alcoholic extract in suitable proportions, the final mixture can be rendered even more sensitive than standard antigen. This is illustrated in Table VII.

TABLE VII

EFFECT OF ADDING SENSITIZING REAGENT TO THE SECONDARY ALCOHOLIC EXTRACT OF HEART MUSCLE

SERUM NO.	SECONDARY ALCOHOLIC EXTRACT PLUS 2 PER CENT SENSITIZING REAGENT TITER 1 + 2.0			STANDARD ANTIGEN TITER 1 + 1.1		
	1	2	3	1	2	3
1	−	−	−	−	−	−
2	−	±	+	−	±	+
3	++	+++	+++	±	+	+++
4	++	++++	++++	+	++++	++++
5	+++	++++	++++	++	++++	++++
6	++++	++++	++++	++	++++	++++
7	+++	++++	++++	+++	++++	++++
8 – 9	++++	++++	++++	++++	++++	++++

It should be mentioned, however, that the precipitates obtained in the positive reactions were unusually fine, suggesting an insufficiency of lipids in the mixture.

SUMMARY

Studies were undertaken to determine whether the by-products obtained in the preparation of antigen for the Kahn test could be utilized for preparing

a stable antigenic solution. An acetone insoluble product of the concentrated ether extractives of heart muscle showed antigenic properties, but when used alone was less satisfactory than standard antigen, and when added to standard antigen did not improve the antigenic qualities of the latter. A secondary alcoholic extract of heart muscle was found to be relatively poor in antigenic properties. An alcoholic extract of the concentrated ether extractives of heart muscle was found to be unfit for direct use as an antigen in the Kahn test, but on the other hand, it was observed that this extract has the property of increasing the sensitiveness of other antigens when mixed with them in suitable proportions. This extract, known as "sensitizing reagent," renders comparatively simple the correction of antigens that are less sensitive than standard. It also renders possible the preparation of antigens more sensitive than the standard.

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2. Kahn, R. L.: Serum Diagnosis of Syphilis by Precipitation, 1924, pp. 68, 121.
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A FILLING DEVICE FOR MICRO-PIPETTES*

By Harry D. Tripp, M.B., Chicago, Ill.

EVERY person who has used pipettes of small caliber is familiar with the tediousness encountered in their usage. The tiny column of fluid is difficult to control by the customary method of applying oral suction by way of a rubber tubing and mouth piece. This method is, of course, also very unsanitary. Several attempts are sometimes necessary in order to have the column stop on the proper calibration. This is especially true with blood counting pipettes.

Other objections to the oral method of filling these pipettes are: (1) By gravitation a certain amount of saliva will find its way into the rubber tubing, and then into the pipette, thus contaminating the pipette and the fluid being drawn into it. (2) Pinching off of the rubber tubing to stop the column may expel a portion of the contents. (3) The danger of infection should some of the bloody mixture, serum or bacterial suspension, be accidentally drawn into the mouth. It is also possible for the rubber tubing to become contaminated after numerous blood counts have been made, and as a rule one does not think to sterilize these tubes before placing them in the mouth. (4) The usual method of blowing one's breath through these pipettes in order to force cleaning fluids through them is not a good one because the breath is the last substance blown through. It condenses on the inner walls of the pipette so that actually the pipette is not clean when the usual cleaning procedure is complete.

To overcome some of the difficulties I have arranged a device adaptable

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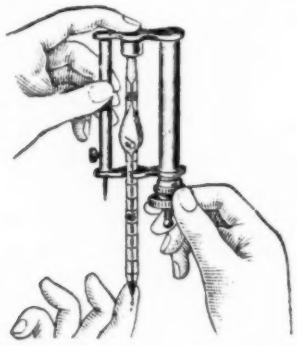
Received for publication, July 23, 1928.

to small pipettes, such as the blood counting pipettes, the Folin-Wu and Byrd pipettes used in blood sugar analyses, and other pipettes as used in the Kahn syphilis test, and those used in making bacterial counts. It is a small, compact device carrying in the same structure an adjustable puncture needle. Pipettes are readily attached and replaced, and facilities for cleaning them are inherent. To operate, one simply holds the device in the hand and manipulates a milled head which perfectly controls the column in the pipette so that specific quantities can be drawn into the pipette or expelled from it. The device is so arranged that after filling the pipette it is held so firmly that agitation of the contents is accomplished by shaking the whole device without removing the pipette.

A few suggestions in the technic of taking blood samples may be helpful as it may appear that this device cannot be used when taking blood from the ear. However, it is not as difficult as it may seem. Usually some inconvenience is experienced because the puncture is made too deep and the blood runs down



the patient's neck or the puncture is not made deep enough and then an attempt is made to express blood by pinching the ear. This is not proper as tissue serum is expressed with the small quantity of blood and if a count is made it will be inaccurate. It is much more convenient to make a sufficiently deep puncture and then collect a few drops of the blood on a clean glass slide which has a small circle drawn on it with a wax pencil. The blood must be placed within the circle to keep it from spreading. To fill a pipette from this then is very easy and much more accurate. The blood will not clot before one fills the pipette, because with the device only a second is sufficient to fill the pipette to the desired mark with blood. A pledget of cotton can be placed on the bleeding ear.

When taking blood from the finger tip I have found it very helpful to loop a small rubber band around the finger tip two or three times after stripping the finger. By doing this only a slight prick will bring blood without further pinching of the finger.

With this device and the few points in technic considerably less experience is required to become efficient in making blood counts and other blood examinations; it simplifies the process of diluting the blood and would appear to be more accurate than the customary method.

5569 Jackson Blvd.

AN IMPROVED ANIMAL CAGE FOR SCIENTIFIC INVESTIGATIONS

By A. Richard Bliss,* Jr., Phm.D., M.D., Memphis, Tenn., and George S. Bliss,† B.S., New York City

THE cages, described herein and used in the laboratories of the University of Tennessee Colleges of Medicine and Dentistry and School of Pharmacy at Memphis during the past college session, have proved very satisfactory. Long experiences with stock cages and with cages constructed in the Shop of the University of Tennessee, all of which showed some marked disadvantages from the standpoints of animal comfort, care, accurate work and costs, resulted in the invention of the cages under discussion.

These cages, which may be built to any desired dimensions, provide isolating compartments for different sizes of dogs, cats, rabbits, monkeys, or other small animals. The cages are constructed so as to furnish maximum ventilation and visibility, while affording complete comfort to caged animals. The high degree of comfort provided is conclusively shown by the fact that large dogs, which have been confined by us in cages of this type 3 feet x 3 feet x 3 feet, have given none of the usual noisy evidences of discomfort and fretfulness.

The cages are constructed of sheet metal (filing cabinet steel) which is covered with baked enamel of desired color, and they present a most pleasing appearance. Fig. 1 presents a general view of one of the cages. The back and sides are of steel plate with heavy wire mesh toppings. The mesh toppings may be made of any desired height. The top of the cage is of steel plate or of wire mesh as desired. The front is a duplex door, the lower unit being a sheet metal door, and the upper a wire mesh door, each provided with a fastening bolt. The sheet metal door carries a large label holder and has an angular upper edge offset laterally inward, against which the wire mesh door closes at the bottom. (See Fig. 2.) This arrangement permits close unobstructed observation of the confined animal, and the placing of food, water, and other materials in the cage with practically no opportunity for the animal to escape.

A water or food tray is carried on the inside of the lower door. (See Fig. 2.) If desired, the tray may be fastened to one of the sides. The tray is attached to threaded rods, the upper ends of which are squared. By simply turning the squared tops of the rods with the fingers the tray is caused to travel up or down to a height which is within comfortable reach of the particular animal under observation, and without the liability of the tray contents being spilled or contaminated by the urine or feces of the animal.

The floor supporting the animal consists of a readily removable and re-

Received for publication, August 4, 1928.

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Fig. 1.—Bliss animal cage.

Fig. 2.—This view shows the duplex door, water or food tray, false mesh bottom and hopper drawer.

placeable false bottom of suitable wire mesh, which permits the free passage of the urinal discharges of the animal. The removable floor rests on a ledge which is provided with a flange directed laterally inward and downward to deflect liquid passing through the mesh floor away from the lower sides of the cage body. (See Fig. 3.)

Under the floor or false bottom is a removable sliding hopper drawer with its inner bottom sloping in all directions toward one outer corner which is provided with a small drain or draw-off cock. Accordingly, all liquid dis-

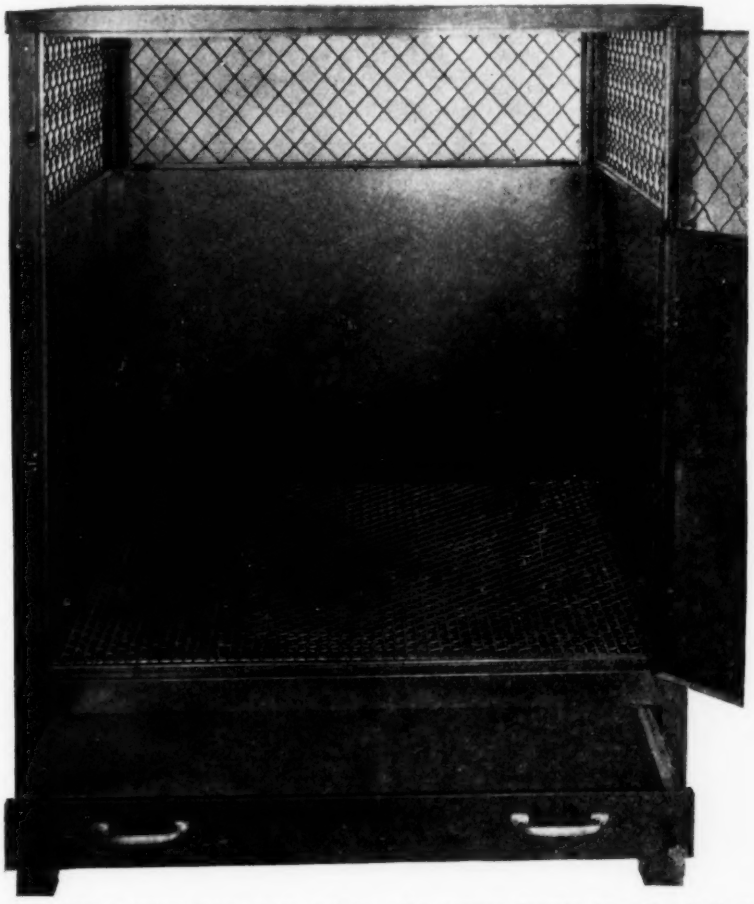


Fig. 3.—View showing false mesh bottom, and hopper drawer carrying drain-cock.

charge from the animal will pass into the hopper drawer where it is accurately and completely collected, and from which it may be completely drawn off at will. The construction material insures against corrosion and contamination of the collected fluids. (See Figs. 2 and 3.)

The legs of the cages are removable so that they may be stacked in tiers. (See Fig. 4.) Castor bases may replace the legs.

The construction and the materials of the cages make them practically indestructible, and permit of easy thorough cleansing and sterilization by dis-

infecting and cleansing agents, boiling water and steam. The weight of the cages permits of easy moving. The cost is surprisingly low.

By an easy modification of the foregoing construction a very inexpensive cage is provided suitable for simple storage and isolation. Full mesh panels

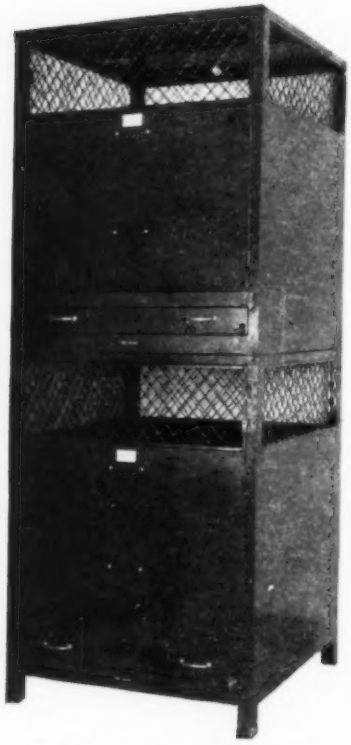


Fig. 4.—Cages stacked in tier.

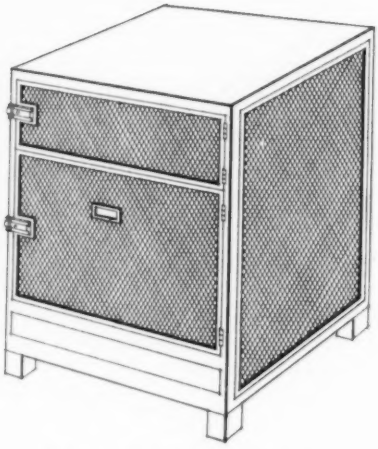


Fig. 5.—Simple storage cage.

may be used for the sides and door of the cage. The back and the top may be made of solid steel or mesh as desired, and the bottom of solid steel in place of mesh. No hopper drawer is then needed. (See Fig. 5.)

A SIMPLE AND EFFICIENT METHOD FOR PERMANENTLY NUMBERING RABBITS*

By K. L. McAlpine, M.A. (Toronto), Glenolden, Pa.

IN THE routine assay of insulin a large number of rabbits are required. The assay is greatly facilitated by injecting two groups of nine rabbits, one with the standard insulin and the other with the unknown. One week later the groups are reversed, so that the one which received the standard now receives the unknown, and vice versa. In this manner variables in the susceptibility to insulin of individual animals tend to balance each other.

The above procedure makes it desirable and almost necessary to number permanently each rabbit, because after the necessary rest period the same

Fig. 1.

animals may be used repeatedly as long as they weigh between 1800 and 2200 gm. Various forms of tags, clips, and rings have been used with more or less success, but often these produced injury to the ear, thereby interfering with the bleeding. Branding with a toothed punch caused infection at times. Numbers printed on with various dyes and pigments, such as methylene blue, fuchsin, safranin, picric acid, indigo carmine, indelible ink, India ink, etc., although brilliant when applied soon faded and became obliterated.

To overcome these difficulties, the following method has been devised and found very satisfactory: The hammer of a discarded electric bell is bent at right angles to the armature and a sewing needle is soldered to the bent portion. A toy transformer attached to the light circuit may be used as a suitable source of current, although any other 6 volt supply is equally efficient. The

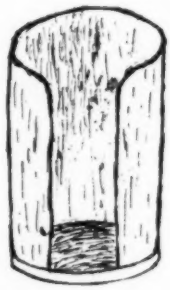
*Received for publication, August 4, 1928.

numbers are written on the inner surface of the ear with India ink, and, while the ink is still wet, the vibrating needle is run along the numbers, thus tatooing the pigment into the skin. This process is very rapid; a rabbit may be tatooed with a 3 digit number in about two minutes. The animal experiences little discomfort and no blood is drawn. The numbers put on by this method are permanent. No infections have been observed.

A USE FOR ETHER CANS*

By Mary B. Cooper, A.B., Syracuse, N. Y.

THE use of quarter-pound anesthetic ether cans to serve as holders for culture tubes dates from the Great War or earlier. The soldered-on cover of the *empty* can is removed by the aid of pliers and of heat from a Bunsen burner.



We have lately adapted the cups thus easily obtained to serve as containers for the small 50 by 13 mm. Petri dishes used for routine blood plates. With tinsmiths' shears a strip about an inch wide is cut out from the rim of the can to the base, care being taken to leave no jagged corners. The plates slip easily in and out of these containers, and are held conveniently during sterilization as well as later when they are filled with media and are in the refrigerator or incubator.

We have found the ether can, which the operating room discards, a useful adjunct to our laboratory.

*From the Laboratory of the General Hospital of Syracuse.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

ALCOHOLISM: Quantitative Study of Acute Intoxication, Bogen, E. Am. J. M. Sc. **176:** No. 2, 153, 1928.

The following methods are proposed by Bogen for the laboratory measurement of alcoholism.

Alcoholic Content of Breath:

Reagent (Anstie). A solution of two-thirds of 1 per cent of potassium bichromate in concentrated sulphuric acid is diluted with an equal quantity of distilled water and a current of air containing the alcohol in gaseous form bubbled through it. The color change, from reddish-yellow to greenish-blue, is then measured by comparison with a series of standards previously made by the addition of known amounts of alcohol to 5 c.c. portions of the reagent mixture in sealed tubes.

Alcohol in urine, blood, spinal fluid, tissues, etc.:

Place 1 c.c. of the specimen in a test tube. A purified air current is bubbled through 5 c.c. of the reagent for ten minutes while the tubes are immersed in a boiling water-bath.

When acetone is present in the unknown solution it must be removed by the addition of 1 c.c. of Scott Wilson's alkaline mercuric cyanide reagent before aeration.

The amount of alcohol present can be determined by color comparison with the standards or, more precisely, by titration as follows:

To the heated and aerated reagent solution add 3 drops of 1 per cent potassium ferrocyanide and titrate with a standard solution of N/30 ferrous ammonium sulphate solution until a deep blue color appears.

Each 2 c.c. of the standard solution less than 10 c.c. required for this titration represents 1 mg. of alcohol when 1 c.c. of the unknown is mixed with 5 c.c. of the reagent.

Interpretation: "border-line," 1 to 2 mg.
Intoxicated, 3 mg. and over.

ALCOHOLISM: Chemical Test For, Naville, F. Rev. méd. de la Suisse Rom. Geneve **48:** 849, 1928.

If a man or animal ingests absolute alcohol to the extent of 2 or 5 gm. per kilo of weight, two hours later there are found 2 to 5 gm. of alcohol per liter of blood. Consequently, the determination of the alcoholic content of the blood from one to three hours after ingestion, will indicate the amount of absolute alcohol which the individual has absorbed per kilo. By multiplying this figure by the weight of the man or animal there will be obtained an amount of absolute alcohol less or equal, never more, to the amount ingested. If the analysis is made more than three or four hours after ingestion, the figures obtained by the calculation will have to be corrected by taking into account the curves obtained by experimentors.

The method of Nicloux is very simple and may be carried out in any laboratory.

Ten c.c. of grams of the substance to be analyzed (blood, urine, organs) are placed in a distilling flask and about 65 c.c. of a saturated solution of picric acid are added. The mixture is boiled and the products of distillation are received in a cooled tube until 20 c.c. have been obtained. Thus 2 c.c. of the distillate contains the alcohol present in 1 c.c. of blood or other product analyzed. When distilled in a vacuum all the alcohol is obtained and if an ordinary still and condenser are used about 90 per cent of the alcohol will be recovered.

Two c.c. of the distillate are placed in a test tube: 2 or 3 drops of a 19 per cent solution of potassium bichromate are added and then 2 c.c. of concentrated sulphuric

acid. The bichromate solution is so made that 1 c.c. is reduced by 5 mg. of alcohol in an acid medium. The mixture is rapidly heated to the boiling point and the bichromate solution is added drop by drop from a micro-burette. The reduction is manifested by the fact that the yellow bichromate is changed into greenish-blue chromium sulphate. As long as the boiling mixture remains slightly green and does not have a frank greenish-blue color there is an excess of bichromate present. Hence, it is easy to calculate the amount of alcohol contained in the product since by proceeding as above the reduction of 1 c.c. of the bichromate solution shows that there were 5 mg. of alcohol in 1 c.c. of the product, consequently the percentage was 5. If the greenish-yellow color persisted after the addition of 0.5 c.c. of bichromate solution, there was 2.5 mg. or 2.5 per cent of alcohol present.

This method is very rapid and the calculation is simple.

CEREBROSPINAL FLUID: The Bicolored Benzoin Reaction (de Thurzo). Meersseman. Soc. de méd. mil. franc. 22: 4, 1928.

REAGENT

Dissolve 0.5 c.c. of 5 per cent sodium carbonate in 40 c.c. of distilled water. Heat to 35° C. and add 5.2 c.c. of absolute alcohol and 0.6 c.c. of a 10 per cent solution of benzoin. To the resultant milky colloidal suspension, maintained at a temperature of 35° add 0.1 c.c. of 1.1 per cent aqueous solution of light green (vert lumiere). After fifteen seconds add successively 0.17 c.c. of 5 per cent alcoholic solution of brilliant fuchsin and 0.36 c.c. of the light green solution. Allow the solution to ripen for twenty minutes. A satisfactory solution is violet, transparent, and has a bluish opalescence.

The test: Set up 13 tubes. Prepare 100 c.c. of 0.3 per cent sodium chloride solution to which is added 1 c.c. of 0.5 per cent sodium carbonate.

In Tube 1 and 2 place 0.5 c.c. of spinal fluid and add to Tubes 2 to 13, 0.5 c.c. of the NaCl solution. Transfer 0.5 c.c. from Tube 2 to Tube 3 and so on to Tube 12. Tube 13 is the control and receives no spinal fluid. The resultant dilutions are 1/1 to 1/2000.

To each tube add 0.5 c.c. of reagent, mix gently and place in a dark place for twenty-four hours. There can then be observed a series of modifications consisting in either precipitation of the colloid particles or changes in color and 10 degrees in the reaction can be distinguished:

0 corresponds to initial, unchanged violet color.
1 corresponds to a reddish violet color.
2 corresponds to a red color.
3 corresponds to a bright red color with slight opalescence.
4 corresponds to minimum precipitation with a reddish violet.
5 corresponds to precipitation and a red color.
6 corresponds to precipitation and a bright red color.
7 corresponds to precipitation and a bluish color.
8 corresponds to precipitation and a green color.
9 corresponds to precipitation and a bright green color.
10 corresponds to precipitation and a white color.

In all cases the precipitate has a reddish or reddish violet color. By using the tube numbers as abscissae and the degrees of the reaction as ordinates it is easy to construct a curve which gives a graphic representation of the results found.

1. Normal cerebrospinal fluids give a minimum change of color in the first 3 tubes, not extending beyond the second degree, a reddish or a reddish-violet color.

2. In general paralysis there is a marked reaction in the first 2 tubes reaching the ninth or tenth degrees, precipitation with a white or bright green color. The curve is less characteristic in treated cases, the maximum of the precipitation corresponding to a less high degree and being displaced toward the right. He observed cases in which the Wassermann reaction was negative after treatment while the bicolored reaction continued to be slightly positive.

3. In tabes the type of the curve is similar to the above but the maximum pre-

cipitation is a little more toward the right. In cerebral syphilis there is observed a change in the second to fourth tubes which does not exceed the third or fourth degree.

4. In meningitis the maximum change occurs between the fifth and tenth tubes. This change reaches high degrees in purulent meningitis and less high degrees in tuberculous meningitis.

The following general conclusions are drawn: a change in color reaching the third degree shows a pathologic fluid. The maximum precipitation occurs in Tubes 5 and 6 in syphilitic disease of the central nervous system and this is the syphilitic zone. The maximum precipitation occurs in higher dilutions in meningitis. He adds that the reaction is qualitative and that the characteristic curves in the different diseases of the central nervous system can be easily distinguished from each other.

TISSUES: Quick Celloidin Method, Rhamy, B. W. Arch. Path. & Lab. Med. 5: 282, 1928.

METHOD

1. Ten per cent formalin, ten minutes.
2. Acetone (depending on size of tissue block), two changes, from one-half to two hours.
3. Acetone, absolute alcohol, ether, equal parts, from one-half to two hours.
4. Acetone, 1 part, thin celloidin, 3 parts, from one-half to two hours.
5. Thick celloidin, twenty-four hours.
6. Mount and harden in full strength denatured alcohol.

After step 3, the method may be varied thus, if desired:

4. Absolute alcohol, ether, equal parts, from one-half to two hours.
5. Thin celloidin, from one-half to two hours.
6. Thick celloidin, twenty-four hours.
7. Mount and harden in 80 per cent alcohol.

SEX HORMONE: Clinical Data Obtained With the Female Sex Hormone Blood Test, Frank, R. T., and Goldberger, M. A. J. A. M. A. 90: 106, 1928.

The reaction consists in obtaining 40 c.c. of venous blood; pouring the unclotted blood in 60 c.c. of pure, not denatured, 95 per cent ethyl alcohol; evaporating to dryness under reduced pressure; triturating the residual powder finely; extracting twice with pure ether; again evaporating to dryness and emulsifying the ether soluble residue in 2 c.c. of sterile distilled water. The emulsion is injected in five divided doses, distributed over a period of ten hours, into a castrated mouse, due precautions being taken to reduce the leakage from puncture holes to a minimum.

A vaginal smear obtained from the mouse in from thirty-six to forty-eight hours is recorded as:

Negative 0 if there is a preponderance of leucocytes in the spread.

Weak +2 if a large amount of nucleated epithelium is present but a few leucocytes persist.

Threshold +3 if the smear contains an excess of epithelial cells, no leucocytes.

Strong +4 if the smear shows only nonnucleated squamous epithelial scales.

Every positive reaction is controlled by daily vaginal spreads examined over a period of ten succeeding days, in order to exclude regeneration of an overlooked fragment of ovary and consequently a mistake due to reestablishment of the spontaneous cyclic reaction.

The conclusions drawn from an investigation of the test in a number of varied conditions were that:

In women with normal sex cycle, more and more of the female sex hormone accumulates in the circulating blood until menstruation sets in.

With the onset of menstruation as well as with the beginning of pregnancy, the hormone disappears from the circulating blood.

The hormone is found in great concentration in the menstrual as well as in the postpartum blood.

The hormone is found in the circulating blood from the twelfth to the fortieth week of gestation.

In functional bleeding a majority of cases show excessive ovarian activity.

Functional overactivity may be demonstrated without excess bleeding (tension) and even in the presence of amenorrhea.

Amenorrheas must be subdivided into (a) a grave type without cycle and into those with (b) subthreshold cycle, (c) self-limited with impending menstruation, and (d) due to persistent corpus luteum. The gravity of the condition depends on the type.

Women who have never menstruated may nevertheless ovulate and their sex tract undergo cyclic changes.

The test, when positive, permits of the determination of sex.

Sterilities probably fall into two classes, the first with normal cycle, the second with depressed function. In the first group other factors besides ovarian function must be taken into account.

Death of the fetus after the twelfth week can be recognized by the blood test.

SMALLPOX: The Laboratory Diagnosis of Smallpox Virus Utilizing the rabbit, Defries, R. D., and McKinnon, N. E. Am. J. Hyg. **8**: 107, 1928.

The reaction in the skin of the vaccine-immunized rabbit following the intradermal injection of smallpox material may be useful in the diagnosis of smallpox, but is so variable, both in occurrence and in character, that accurate interpretation is often impossible.

The corneal reaction is specific, but positive results have been found by the authors in only about one-half of the tests when known smallpox material was used.

The intradermal injection of smallpox material into normal rabbits evokes regularly a definite, specific lesion.

The development and retrogression of the lesion produced by the intradermal injection of smallpox material into the normal rabbit differs markedly from the course of the lesion similarly produced in the vaccine-immunized rabbit.

The repeated intradermal injection of smallpox material into rabbits produces in them a definite immunity against vaccine virus.

A laboratory test for smallpox virus is found in the development of the typical lesion following intradermal inoculation of smallpox material in the normal rabbit, contrasted with the response following a similar procedure in the vaccine-immunized rabbit.

SPINAL FLUID. Further Data On the Boltz Test, Cady, L. D. Arch. Neurol. and Psychiat. **18**: 565, 1927.

The Boltz test is made by placing 1 c.c. of spinal fluid in a Wassermann test tube and adding 0.3 c.c. of acetic anhydride drop by drop. The contents of the tube are then shaken, and 0.8 c.c. of concentrated sulphuric acid is added drop by drop. The tube is again shaken and observed for about five minutes, when a blue-pink or lilac color will develop in positive general paralytic fluids. Boltz believes that the test is of diagnostic and prognostic significance in neurosyphilis.

The Boltz test is a modification of the Liebermann cholesterol test, but these fragmentary observations fail to show that cholesterol is in any way responsible for the positive reaction. The modified Boltz test has been used on 799 specimens of spinal fluid. Positive reactions were found in from 79 to 94.6 per cent of patients with neurosyphilis when the test had not been rendered negative by treatment. The modified test was positive in 42.4 per cent of 264 other nonsyphilitic neuropsychiatric patients. It was found positive in 27.2 per cent of seventy-seven patients with constitutional diseases. This occurred for the most part in patients with arthritis of the spine. The original test seems of some value in indicating the presence of abnormal processes in the meninges and the central nervous system, and promises to be of a value at least equal to other routine nonspecific chemical tests. The modified test may have considerable value in the control of treatment of neurosyphilis. These observations indicate that, whenever the test is used routinely, the original and the modified technic should be used on each specimen of spinal fluid examined.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.

*Reagenzien und Naehrboden**

THE collection of reagents is divided into those for qualitative tests, for quantitative tests, for physiologic chemistry, and for microscopic technic. Then follows the culture media, preserving and hardening fluids, and miscellaneous formulas.

The authors are two German pharmacists, and they apparently know little or nothing about modern American methods. For example, there are more than 400 formulas for physiologic chemistry and yet there is nothing of modern blood chemistry or the micro-methods. There are about 250 formulas for culture media but neither E. M. B. agar nor Russell's Double Sugar Medium is given.

However, it is a right complete collection of German formulas and includes many which sound right strange to us.

Die Wasserstoffionenmessung†

THIS is a small book of only 140 pages which attempts to give the essentials of the determination of hydrogen-ion concentration in the simplest possible manner so that those without special training in physical chemistry or mathematics can understand it. After preliminary chapters on solutions ionization and buffers the author explains the process of determining the hydrogen-ion concentration both by the use of indicators and by electrical methods for the worker who has no other help than this little book. Clark and Lubs indicators and other American work are given recognition.

Principles of Public Health Engineering‡

THIS volume is the outgrowth of a course in public health engineering given by the author at Columbia University and is intended for the medical health officer without engineering training, and for the sanitary engineer without knowledge of public health principles. "In brief the book attempts to furnish the public health background to the conventional sanitary engineering course, and an engineering viewpoint to the medically trained man doing public health work."

In this aim the book is commendably successful and serves a useful purpose. For those interested in the subject there are lists of collateral reference works at the end of each chapter.

The book can be recommended to those to whom it is addressed.

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*Reagenzien und Naehrboden (Reagents and Culture Media—A Collection of the Most Important and Reliable Formulas for the Laboratory). By Boehm and Dietrich, Urban and Schwarzenberg, Berlin.

†Die Wasserstoffionenmessung (The Measurement of Hydrogen-Ion Concentration). By Gunther Lehmann. Published by Johann Ambrosius Barth, Leipzig.

‡The Principles of Public Health Engineering. By E. B. Phelps, Professor of Sanitary Science, Columbia University. Cloth, 265 pages, 35 figures. The Macmillan Co., New York.

NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

*Growing Up**

MODERN educators and modern parents are agreed upon the wisdom, the benefits, and the necessity of imparting to children a correct knowledge of sex life. It is one thing, however, to have knowledge and another to be able to impart it, and gifted, indeed, are they who can speak to children on these matters in a tactful, understandable, and inoffensive way.

Because of this inherent difficulty there is a tendency to pass on this responsibility and for each, the parent, the teacher, the doctor and so on, to evade this duty, in large measure for lack of a satisfactory way to go about it.

Of all the books which have been written, there are none so commendable, none so valuable, and none so entirely satisfactory as this one by Karl de Schweinitz.

There is nothing in it which smacks of "now children"; there is nothing to arouse suspicion that something is being suppressed; nothing to awaken unsatisfied curiosity; nothing to cause embarrassment to child or parent; nothing incomprehensible to a child's mind. The book is marvelously natural, entirely matter-of-fact, of absorbing interest and, withal, accurate and complete. There is no glossing over, no omissions, the book admits of no criticism for, and this, indeed, is its main virtue, that without the slightest suggestion of the teacher, without any portentous solemnity, it leaves the child reader undisturbed in the understanding that mating and birth are natural, seemly, and good things concerning which there should be no false cloak of shame or prudery.

To the parent and teacher, and to the harassed doctor consulted upon how such teaching should be done, this book will be of inestimable value.

There is only one regret, that there is no way in which it could be placed in the hands of every parent and every child.

Medical Bacteriology†

THIS book is intended primarily for the medical student and practitioner, its purpose being to present simply in a practical way the accepted relation of bacteria to medicine.

It is divided into two parts. Part I, Descriptive Bacteriology, presents in a simple yet relatively comprehensive manner, a discussion of the properties of bacteria in relation to disease, the ordinary methods of cultivation, staining, etc., available for their study and identification and in various serologic and pathogenicity tests. There is a short but useful chapter on common contaminating organisms following which the bacteria of ordinary pathogenicity are described.

Fungi, filterable viruses, protozoal diseases and helminth infections are next described. There is a short but practical chapter on bacterial preparations: vaccines, sera, etc.

In Part II, Applied Bacteriology, the uses of bacterial preparations are discussed. Because the bacteriologic examination may be, and, as the practical experience of any pathologist will testify, often is, ruined by a faulty collection of the specimen, a chapter is devoted to this.

Then follows a discussion of laboratory aids in the diagnosis and treatment of various diseases which are arranged in alphabetical order. The final chapter discusses succinctly the bacteriology of water, milk and food.

This little book should be eminently useful to those to whom it is addressed.

Anthelmintics and Their Uses‡

IN THE preface to this distinctly useful book, the authors comment very pertinently upon the fact that helminths form a separate and distinct group of organisms and that the drugs used to combat them are also a distinct group since they are seldom used for other purposes.

---

*Growing Up. By Karl de Schweinitz. Cloth. 111 pages, 30 illustrations. The Macmillan Co., New York.

†Medical Bacteriology, Including Elementary Bacteriology. By L. H. Whitby. Cloth. 320 pages, 75 illustrations. J. and A. Churchill, London.

‡Anthelmintics and Their Uses in Medical and Veterinary Practice. By R. N. Chopra, M.D., Professor Pharmacology, Calcutta, School of Tropical Medicine and Hygiene, and A. C. Chandler, Ph.D., Professor of Biology, Rice Institute, Houston, Texas. Cloth. 291 pages. Williams and Wilkins Co., Baltimore, Md.

Because of these facts, because these organisms differ in their reaction to drugs, because many of the details concerning anthelmintics and their uses appear in relatively inaccessible places, and because, by virtue of the structural and physiologic diversity of helminths there can be no universal anthelmintic, this book has been written.

It is divided into three general sections in the first of which the general considerations applicable to the subject are discussed together with a discussion of the correlation between chemical composition and anthelmintic action.

Section II is devoted to a discussion of anthelmintics acting on parasites in the gut and Section III considers those used against somatic parasitic infections.

The literature of the subject has been freely consulted and evaluated in the light of the authors' experience.

The book, stated to be the first of its kind, should fill a very useful place in the practitioner's library.

*A Textbook of Biologic Assays**

THIS, the second edition of a well-known and extremely practical manual, has been so extensively revised as to have been practically rewritten. It may be regarded as an authority in its field and highly commended to those engaged or interested in pharmaceutical assay or drug standardization.

Laboratory Manual of Physiological Chemistry†

THIS manual is intended primarily for the teaching of students of biochemistry and presents in nine chapters a well ordered series of exercises including the accepted modern methods, especially quantitative, of physiologic chemistry. It is interleaved for the insertion of notes and should prove acceptable to both teacher and student.

Clinical Laboratory Procedures‡

THIS is essentially a compendium of the various methods used in the author's laboratories, the book being interleaved for the convenience of the user.

The Determination of Hydrogen Ions§

NEITHER this book nor its author requires any introduction. Dr. Clark's pioneer studies in the study of hydrogen-ion determination and his relation to the development of this field of biologic chemistry are sufficient in themselves to assure the authenticity and authority of this volume.

The present edition embodies a comprehensive review of all the advances in technic and all the applications of hydrogen-ion determination to biologic studies in health and disease.

Despite the complexities of the subject the style is exceedingly clear and, at the same time happy and scholarly. It is a volume to be owned and read with pleasure as well as with immeasurable profit.

It is a welcome innovation, indeed, to read a volume the nature of whose subject would lead the reader to apprehend a ponderous style, enlivened by chapter mottoes, as it were, both grave and gay and even from "Alice in Wonderland," but to the point, nevertheless!

Dr. Clark and the publishers are to be congratulated and the reader as well that such a book awaits his perusal and study.

*A Textbook of Biologic Assays. By P. S. Pittenger, Ph.G., etc. Instructor in Biologic Assay, Phila. College of Pharmacy, etc. Second edition. Cloth, 373 pages, 153 illustrations. P. Blakiston's Son & Co., Philadelphia.

†Laboratory Manual of Physiological Chemistry. By M. Bodansky, Associate Professor Biological Chemistry and M. S. Fay, Adjunct Professor Biological Chemistry, University of Texas. Cloth. 234 pages, 9 figures. John Weleg and Sons, Inc., New York.

‡Clinical Laboratory Procedures. By G. L. Rohdenburg, M.D., Director of Laboratories, Lenox Hill Hospital. Cloth. 266 pages. The Macmillan Co., New York.

§The Determination of Hydrogen Ions. By W. M. Clark, Professor of Physiological Chemistry, Johns Hopkins University, Ed. 3. Cloth. 717 pages, numerous tables. Williams and Wilkins Co., Baltimore, Md.

The Journal of Laboratory and Clinical Medicine

VOL. XIV ST. LOUIS, MO., JUNE, 1929 NO. 9

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EDITORIALS

The Sanitary Significance of Lactose-Fermenting Bacteria Not Belonging to the B. Coli Group

THE determination of the potability of water, a matter of great sanitary importance, has always been the subject of some confusion in the lay mind. There are many, for example, who believe implicitly that all that is necessary is a single bacteriologic study, failing to appreciate that such an examination applies only to the water at the time of examination; that it offers no absolution for the sins of the past and no guarantee for the future; and that without interpretation in terms of a sanitary survey, it is but a reed to lean upon.

There is a popular idea, also, that running water, if not always pure, will purify itself in its course, an idea which investigations of the U. S. Public Health (Bull. 173) have shown to be quite erroneous.

These fallacious conceptions at times render the interpretation to the public of bacteriologic water examinations a matter of difficulty, especially as the subject itself has inherent difficulties demanding careful consideration.

In a general way, without entering into a discussion of the minutiae of the subject, bacteriologic examinations are largely concerned with the detection of B. coli, and much interest, therefore, attaches to those organisms, which may be a source of confusion.

Believing that the sanitary importance of such bacteria, lactose fermenters not belonging to the colon group, has been somewhat overlooked, Greer[1] and his collaborators have recently restudied the question in some detail based upon examinations of the Chicago water supply.

The most common lactose-fermenting bacteria encountered in water are Cl. Welchii, B. aerosporus, Streptococcus fecalis, and members of the mucosus capsulatus group.

In the Chicago water supply these and related bacteria were shown to have a seasonal variation and to vary in raw and treated water, so much so as to render valueless, during many months of the year, the forty-eight-hour presumptive test of treated water as an index of B. coli. In Chicago, therefore, the presumptive test must be carried to completion to avoid error during the greater part of the year.

This fact is of practical importance and brings up a number of questions in connection with the use of lactose-broth for the isolation of any single organism.

A review of the literature shows that B. coli is outgrown and inhibited in culture media by several bacteria found in water. The experimental work of Greer and his associates shows that: a) B. coli may be outgrown by S. fecalis, Cl. Welchii, and Ps. pyocyaneous in forty-eight hours; b) B. aerosporus is outgrown by all these organisms in both twenty-four and forty-eight hours; c) If B. coli is present in these mixtures, it can usually be isolated in twenty-four hours from tubes showing gas within this time; d) Combinations of organisms found in water may give rise to gas production which is not due to the presence of B. coli.

A study of the literature reveals that only two of the lactose fermenting organisms other than B. coli, Cl. Welchii and the Friedlander group, are commonly associated with pathologic conditions in man. Ps. pyocaneus may or may not be pathogenic when ingested. Greer and his coworkers found it usually associated with B. coli, and believe that its potential properties warrant the condemnation of a water from which it is isolated.

The question of the survival of microorganisms in water is of marked importance.

Water is not a satisfactory menstruum for the survival of intestinal bacteria, and the hydrogen ion concentration, temperature, sunlight, and sedimentation all play an important rôle in determining the survival of intestinal bacteria in water.

B. coli usually survives longer than B. typhosus, S. fecalis perishes rapidly, while the evidence suggests that spore-formers survive the longest. Due to

the variety of factors influencing the results, definite time tables of survival are difficult to compile. Each water with the concomitant circumstances is, to some extent, a law unto itself.

The results of this study are of interest in connection with the technic of bacteriologic water examination, the present tendency of which has been toward the use of selective media and methods adapted to the detection of B. coli to the exclusion of other bacteria.

The work of Greer and his associates leads them to believe that the best combination of mediums and methods include lactose broth for the presumptive test, eosin methylene blue for the confirmatory test, and lactose broth and agar slants for the completed test.

Their conclusions may be thus summarized:

Besides B. coli, S. fecalis, Cl. Welchii, and Ps. pyocyaneus may all be of sanitary importance in water examination, S. fecalis probably having as much sanitary significance as B. coli, while Cl. Welchii and Ps. pyocaneus are of less significance, and B. aerosporus of no significance.

While not suggesting that any of these organisms are any better indexes of fecal pollution than the fecal type of B. coli, they stress the fact that there are lactose-fermenting bacteria in water other than the colon-aerogenes group, some of which have sanitary significance and cannot be ignored.

They suggest, therefore, that it may well repay routine control laboratories to discover what lactose-fermenting organisms other than B. coli are present in their water supplies; and to study and determine the potentialities of the types of pollution present, especially as such investigation may reveal the source of false negative and positive results encountered in the routine examination for B. coli.

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—*R. A. K.*

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FRIDAY, JULY 5, 1929, 9 A.M.

Call to Order
Short Business Session.

Scientific Program

The Effect of the Presence of Bile on the Agglutination Reaction. By Ruth Gilbert, M.D., and Marian B. Coleman, B.S., Albany, N. Y.

Tularemia (Francis' Disease): A Report of Ten New Cases with an Analysis of Sixty-three Cases Occurring in Dayton, Ohio. By Walter M. Simpson, M.D., Dayton, Ohio.

The Routine Use of the Photoelectric Hemoglobinometer. By A. H. Sanford, M.D., and Charles Sheard, Ph.D., Rochester, Minn.

Polycythemia (Patent Foramen Ovale). By Herman Spitz, M.D., Nashville, Tenn.

Acute Diffuse Myelitis Following Intravenous Injection of Neoarsphenamine. By Ernest Scott, M.D., and H. L. Reinhart, M.D., Columbus, Ohio.

Spontaneous Meningeal Hemorrhage. By Frederick H. Lamb, M.D., Davenport, Iowa.

FRIDAY, JULY 5, 1929, 2 P.M.

Symposium on Undulant Fever

Undulant Fever in Man: A Clinical Analysis of Thirty-three Cases. By A. S. Giordano, M.D., and R. L. Sensenich, M.D., South Bend, Indiana.

Discussion on Undulant Fever. By Walter M. Simpson, M.D., Dayton, Ohio.

The Pathogenicity for Monkeys of Brucella Abortus. By Merrill J. King, M.D., Mount MacGregor, New York. (By invitation.)

The Etiology and Diagnosis of Undulant Fever in the United States. By Charles M. Carpenter, M.D., and Ruth Book, Ph.D., Ithaca, New York. (By invitation.)

Some Observations on the Agglutination of B. Abortus. By Frank B. Lynch, Jr., M.D., and Annette M. Callan, Philadelphia, Pa.

Notes on the Bacteriology of the Brucella Group. By K. J. Meyer, M.D., San Francisco, Calif., to be read by J. C. Geiger, M.D., San Francisco, California. (By invitation.)

FRIDAY, JULY 5, 1929, 7 P.M.

Round Table Discussion

Virtuosity in Clinical Pathology. By Philip Hillkowitz, M.D., Denver, Colo.

Problems. By W. G. Gamble, Jr., M.D., Chicago, Illinois.

The State Laboratory Problem. By Edward F. Cooke, M.D., Houston, Texas.

The Hospital Situation.

A. Economics. By Herman Spitz, M.D., Nashville, Tennessee.

B. Scientific. By Robert A. Keilty, M.D., Washington, D. C.

C. Statistical. By Philip B. Matz, M.D., Washington, D. C.

D. Relation to the American College of Surgeons. By J. J. Moore, M.D., Chicago, Ill.

Postmortems. By Herbert R. Mills, M.D., Tampa, Fla.

Is the Cost of Laboratory Work Too High? By Robert F. E. Stier, M.D., Spokane, Wash.

The Clinical Pathologist in the Rural Hospital. By C. W. Maynard, M.D., Pueblo, Colo.

SATURDAY, JULY 6, 1929, 9 A.M.

Gingivitis V: The Character of the Exudate in Gingivitis. By Robert A. Keilty, M.D., Washington, D. C.

The Tuberculous Cavity. By Alfred Blumberg, M.D., Oteen, N. C.

Oxalic Acid as a Reagent for Isolating Tubercle Bacilli and a Study of the Growth of Acid-fast Non-Pathogens on Different Mediums with Their Reaction to Chemical Reagents. By H. J. Corper, M.D., and Nao Uyei, Ph.D., Denver, Colo.

A Recently Isolated Bacillus of the Hemophilic Group. By Frank W. Hartman, M.D., and Edna Jackson, M.S., Detroit, Mich.

Immunological Specificity of Green Producing Streptococci Having Elective Localizing Power as Isolated in Various Diseases. By E. C. Rosenow, M.D., Rochester, Minn.

Milk Borne Rabies. By E. R. Mugrage, M.D., Denver, Colo.

Observations on Intestinal Protozoiasis. By Rawson J. Pickard, M.D., San Diego, Calif.

SATURDAY, JULY 6, 1929, 2 P.M.

Pathology of the Reticulo Endothelial System. By Zera E. Bolin, M.D., San Francisco, Calif.

Reticulocytes, Their Identification and Significance. By C. L. Spohr, M.D., and Alice Bustine, Columbus, Ohio.

Improved Colorimetric Procedures for the Quantitative Estimation of the Proteins of the Cerebrospinal Fluid. By Philip B. Matz, M.D., and Nathan Novick, Washington, D. C.

Quantitative Microscopic Urinalysis. By William G. Exton, M.D., Newark, N. J.

New Quantitative Clinical Methods for the Junior Scopometer.

1. Protein in Urine
2. Protein in Blood
3. Protein in Spinal Fluid
4. Globulin in Urine
5. Sugar in Urine
6. Sugar in Blood
7. Urea in Urine
8. Ammonia in Urine
9. Creatinine in Urine
10. Sulphur Partition in Urine

By William G. Exton, M.D., Anton R. Rose, Ph.D., Fred Schattner, Ph.D., and P. V. Wells, D.Sc., Newark, N. J. (To be read by Title.)

Embryonal Carcinoma of the Testicle. By L. W. Larson, M.D., Bismarck, N. D.

Malignant Tumors of the Testicle. By O. A. Brines, M.D., Detroit, Michigan.

SATURDAY, JULY 6, 1929, 7 P.M.

Annual Banquet

Presidential Address. By Dr. Frank W. Hartman, Detroit, Mich.

Address. By Dr. Richard B. Dillehunt, Dean of the Medical School of the University of Oregon, Portland, Ore.

Address. By Dr. Cyrus C. Sturgis, Professor of Medicine at the University of Michigan, Ann Arbor, Mich.

Presentation of the Ward Burdick Research Award.

MONDAY, JULY 8, 1929, 9 A.M. TO 12 AND 2 P.M. TO 5 P.M.

Business Session

Call to Order.

Reading of Minutes.

Unfinished Business.

Reports of Committees:

Executive Committee—Dr. A. H. Sanford, Chairman, Rochester, Minn.

Publication Committee—Dr. John A. Kolmer, Chairman, Philadelphia, Pa.

Editorial Committee—Dr. T. B. Magath, Editor-in-Chief, Rochester, Minn.

Committee on Registration of Technicians—Dr. Philip Hillkowitz, Chairman, Denver, Colo.

Public Relations Committee—Dr. Edward F. Cooke, Chairman, Houston, Texas.

Service Bureau Committee—Dr. H. J. Corper, Chairman, Denver, Colo.

Research Committee—Dr. A. S. Giordano, Member, South Bend, Ind.

Committee on Exhibits—Dr. C. H. Manlove, Chairman, Portland, Ore.

Committee on Necrology—Dr. J. H. Black, Chairman, Dallas, Texas.

Report of Board of Censors—Election of New Members.

New Business.

Report of Nominating Committee—Nomination of Officers.

Election of Officers.

Induction of Officers.

Adjournment.

Scientific Exhibits

Apparatus. By B. W. Rhamy, M.D., Fort Wayne, Ind.

Mounted Specimens of Pathology. By C. H. Manlove, M.D., Portland, Ore.

Razor Section Method. By B. T. Terry, M.D., Rochester, Minn.

The Problem of Gingivitis. By Robert A. Keilty, M.D., Washington, D.C.

Scientific Exhibit. By Alfred Blumberg, M.D., Oteen, N. C.

Specimens and Pictures of a Case of Coccidioidal Granuloma. By D. Schuyler Pulford, M.D., Woodland, Calif.

Personals

In the Bulletin of the American Hospital Association for May, 1929 we read that Dr. J. J. Moore of Chicago spoke before the Indiana Hospital Association in Indianapolis, April 11th, on the financial phase of the hospital laboratory.

That the specialty of clinical pathology is far from foundering on the rocks is evidenced by the fact that a number of our men are taking summer voyages to Europe. Dr. Frederic E. Sondern, New York City, is making his annual pilgrimage abroad. Dr. Alvin G. Foord of Buffalo, N. Y., is leaving for the Continent the last of May. Dr. A. H. Schade, Toledo, Ohio, plans a trip for the fall.

Dr. W. G. Gamble, Jr., of Chicago is making a strenuous effort among the members of the American Society of Clinical Pathologists to charter a special car from Chicago to the Portland Convention.

Dr. William J. Deadman, President of the Hamilton Medical Society, will, in his official capacity, act as host to the Ontario Medical Association Convention during the latter part of May.

The increasing importance of the Laboratory as an aid to diagnosis is demonstrated in the comparatively frequent papers on the subject at various state meetings. At the meeting of the Ontario Medical Association Dr. S. H. McKee of Montreal will speak on the "Relation of the Clinical Laboratory to Clinical Ophthalmology."